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# YEAR BOOK 56

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July 1, 1956—June 30, 1957

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CARNEGIE INSTITUTION OF WASHINGTON  
WASHINGTON, D. C.

1957



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# STAFF

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## ASTRONOMY

### MOUNT WILSON AND PALOMAR OBSERVATORIES

813 Santa Barbara Street, Pasadena 4, California

Mount Wilson Observatory organized in 1904; George E. Hale, Director 1904–1923, Honorary Director 1923–1936; Walter S. Adams, Director 1924–1945. Unified operation with the Palomar Observatory of the California Institute of Technology began in 1948.

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### GEOPHYSICAL LABORATORY

2801 Upton Street, N. W., Washington 8, D. C.

Organized in 1906, opened in 1907; Arthur L. Day, Director 1909–1936; Leason H. Adams, Acting Director 1936–1937, Director 1938–1952; George W. Morey, Acting Director 1952–1953.

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Hans P. Eugster		

### DEPARTMENT OF TERRESTRIAL MAGNETISM

5241 Broad Branch Road, N. W., Washington 15, D. C.

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E. H. Creaser	J. J. Leahy	Irena Z. Roberts
W. C. Erickson	F. T. McClure **	H. Weaver
K. L. Franklin		

\* Retired June 30, 1957.

‡ Resigned in 1956.

§ On leave of absence.

|| Term of appointment completed in 1956.

¶ Resigned in 1957.

\*\* Term of appointment completed in 1957.



**BIOLOGICAL SCIENCES****DEPARTMENT OF PLANT BIOLOGY***Stanford, California*

Desert Laboratory, opened in 1903, became headquarters of Department of Botanical Research in 1905; name changed to Laboratory for Plant Physiology in 1923; Daniel T. MacDougal, Director 1906–1927. Reorganized in 1928 as Division of Plant Biology, including Ecology; Herman A. Spoeher, Chairman 1927–1930 and 1931–1947, Chairman Emeritus 1947–1950. Name changed to Department of Plant Biology in 1951.

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Malcolm A. Nobs  
James H. C. Smith

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Wolf Vishniac

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F. J. F. Fisher  
Paul H. Latimer  
Kazuo Shibata

*Investigator Engaged in  
Post-Retirement Studies*

Jens C. Clausen

**DEPARTMENT OF EMBRYOLOGY***Wolfe and Madison Streets, Baltimore 5, Maryland*

Organized in 1914; Franklin P. Mall, Director 1914–1917; George L. Streeter, Director 1918–1940; George W. Corner, Director 1941–1955.

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*Special Investigators*

Vincent J. De Feo  
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*Research Associates*

Arthur T. Hertig  
Chester H. Heuser  
Samuel R. M. Reynolds

**DEPARTMENT OF GENETICS***Cold Spring Harbor, Long Island, New York*

Station for Experimental Evolution opened in 1904; name changed to Department of Experimental Evolution in 1906; combined with Eugenics Record Office in 1921 to form Department of Genetics. Charles B. Davenport, Director 1904–1934; Albert F. Blakeslee, Director 1935–1941.

Milislav Demerec, *Director*

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Berwind P. Kaufmann  
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*Special Investigators*

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Helen Gay  
Sheila Howarth ‡  
Etta Käfer §  
Andrej W. Kozinski

Ernest L. Lahr  
Joseph D. Mandell  
Atif Sengün ‡  
Jun-ichi Tomizawa  
Sibergina Wagenaar ‡

\* Resigned September 21, 1956.

‡ Term of appointment completed during the report year.

§ Resigned during the report year.

## STAFF *Continued*

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### ARCHAEOLOGY

#### DEPARTMENT OF ARCHAEOLOGY

*10 Frisbie Place, Cambridge 38, Massachusetts*

Department of Historical Research organized in 1903; Andrew C. McLaughlin, Director 1903–1905; J. Franklin Jameson, Director 1905–1928. In 1930 this Department was incorporated as a section of United States history in a new Division of Historical Research; Alfred V. Kidder, Chairman 1930–1950. Name changed to Department of Archaeology in 1951.

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J. Eric S. Thompson

#### RESEARCH ASSOCIATES *of Carnegie Institution of Washington*

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Louis B. Flexner, University of Pennsylvania  
Willard F. Libby, University of Chicago  
Paul W. Merrill, Mount Wilson Observatory  
John von Neumann, || Institute for Advanced Study  
Hans Ramberg, University of Chicago  
C. E. Tilley, Cambridge University  
Evelyn M. Witkin, State University of New York

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\* Retired in 1956.

‡ On leave of absence.

§ Retired in 1957.

|| Died February 8, 1957.

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\* Retired June 30, 1957.





CARNEGIE INSTITUTION OF WASHINGTON

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*REPORT of*  
*THE PRESIDENT*



# CARNEGIE INSTITUTION OF WASHINGTON

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## REPORT OF THE PRESIDENT

It does not matter what a man does; so long as he does it with the attention which affection engenders, he will come to see his way to something else. After long waiting he will certainly find one door open, and go through it. He will say to himself that he can never find another. He has found this, more by luck than cunning, but now he is done. Yet by and by he will see that there is one more small, unimportant door which he had overlooked, and he proceeds through this too. . . . Then after years—but probably not till after a great many—doors will open up all round, so many and so wide that the difficulty will not be to find a door, but rather to obtain the means of even hurriedly surveying a portion of those that stand invitingly open.—Samuel Butler in

*Alps and Sanctuaries of Piedmont and the Canton Ticino*

What is a Golden Age? What echoes of the Age of Pericles, of Renaissance Italy and the Low Countries and Scandinavia, of Elizabethan England, mark each as a flood tide in the vast, slow surge of human intellectual development? Will such flood tides come again?

It is interesting to notice, as James Joll has recently done, some of the characteristics that these ages had in common. All of them were times of fervent intellectual excitement, when major new creations and new experiences and viewpoints were just coming to wide notice and were on the threshold of general acceptance. In all of them one can sense a vigorous address to new ideas—when indeed opening vistas, half-seen, made of ideas precious coin. All of them were eras of some physical security and at least some political and organizational stability. But in all of them, too, stability and security were far from complete, and there is the flavor of a partnership of disorder and hazard with vitality and creativeness. None of them, clearly, were especially “comfortable” times in which to live, in the sense that static and secure environments may be comfortable. And yet, as Joll has significantly pointed out, men knew that they were living in great times. The adventurous in all these periods would probably have admitted—perhaps bitterly resented—the danger and the insecurity and the muddled opacity of their days. But if hard pressed probably no one of them would have admitted a wish to be born in any other era.

Will such times come again? It is hard to imagine that they will not. Indeed, though we hear our own age criticized as static and as anti-intellectual often enough, perhaps we ourselves are the restless, insecure, anxious, vital participants in an era of contemporary intellectual development that other men sometime, somewhere, may well look back upon as golden too.

If we are in fact witnessing the earlier phases of another era of turbulent change, when viewpoints shift rapidly and radically, serving as the anvils for new ideas, we must expect it to differ in many respects from similar periods in the past. One striking difference will be that we cannot hope to localize it geographically. The interlocked character of the present world, the growing

similarity of all its cultures, the universality of its communication, must make meaningless any such designation as an Athenian or an Elizabethan age. But possibly we can characterize it in terms of subject matter, of the loci of ideas with which it is especially concerned. Prominent among such domains, clearly, will be the natural sciences.

Such a situation is not new, of course, for ideas in these fields have figured in the conceptual revolutions of all the Golden Ages. Aristotle and Plato and Socrates all lived in or close to the times of Periclean Athens; Galileo and Copernicus, Da Vinci and Vesalius were of Renaissance Italy, Francis Bacon and William Harvey were of Elizabethan England. But, as any new Golden Age will be impossible to localize geographically, so will its contributions of scientific ideas be derived over a wide and sometimes rather inchoate intellectual front. We can already see vivid examples of this development. And if we compare the current product of the natural sciences over the world for any single year, not only in volume and diversity of source but in scope of consequences, with the whole product of a Periclean Age, we are all but forced to conclude that, half-unknowing, half-unrealizing, we are living in proximity to one of the most astounding Golden Ages of all time.

Surely our age shares many characteristics with the earlier golden times. There is the relative physical safety and comparative political stability over much of the face of the globe. There is the wide feeling of insecurity, the deep-lying anxiety, the sense of confusion, not unlike the earlier times in its general character even though, to us at least, its causes seem far more complex, more massive, more intractable. But there is likewise the same intense concern with new ideas and new concepts, the same eagerness for widened vistas of understanding. And there is another and an important characteristic of such times in which our age also seems typical.

The classical Golden Ages were intensely concerned with the problem of communicating the new ideas that were being born in such profusion. In all of them there was a preoccupation with the problems of education. All of them were times for the establishment of special schools of thought and of great centers of learning, from the Peripatetics to the College of Merton to Padua to Paris. In this characteristic, too, our age resembles the earlier ones, even if groping, as yet, toward developments of educational concepts comparable to theirs.

In the field of communication in its most general sense, however, our age confronts a challenge of almost new dimensions, perhaps nowhere more poignant than in the natural sciences.

Diversity of approach is the very lifeblood of the scientific effort. Science enlists men of the most unlike temperaments and talents. It unites workers whose gifts are primarily descriptive with workers whose understanding and



approaches comprehend symbolism and techniques of the most abstruse and involved character. Bonded in a common effort are men whose talents are primarily synthetic with men so keenly and entirely analytical that synthesis may have little meaning for them. United are investigators of deeply theoretical bent with investigators of primarily mechanical skills. And since in every investigation the observer and his "real" world are in some sense in equilibrium, scientists with divergent gifts and interests, even when concerned with the same problem, necessarily labor in partly different universes.

It is not only the observers that differ widely in their characteristics, under the common rubric of scientists. The subject matter diverges even more. Scientific disciplines vary enormously among themselves in their degree of sophistication and in their intricacy. The attitudes, the modes, the ways of "picking up the stick," to use Butterfield's expressive phrase, even the underlying attitudes and aspirations of the work, may be almost unrecognizably different in a mature, well cultivated, highly differentiated discipline on the one hand and in an exploratory one, still in its primarily descriptive phase, on the other. And though the newer disciplines must always be in some measure rooted in the old, and though it is probable that the tested approaches of older fields always have some relevance for the newer ones, the transfer is far from literal. To accomplish it successfully requires talent and sophistication in the investigator, and, above all, that wisdom and sense of proportion that can come only from broad experience and a flexible viewpoint.

These profound diversities among investigators and within the structure of science are characteristic and immeasurably precious. But they also harbor all the dangers of fragmentation and pose the most serious challenges to communication within the very core of the scientific effort. The compartmenting of subject matter is a constant threat to the unity of science, and many factors promote it. Mere growth of vocabulary and specialization of terminology in a given field—to the point where its jargon becomes unintelligible not only to the layman but even to an investigator working in a nearly adjacent area—raise practical barriers to understanding, barriers that may be formidable.

But there is a more serious aspect to such failures of communication. Words are basically the coin of ideas, and to some degree their generators—never entirely their consequences. So it is not uncommon to find that not only the words but also some basic concepts governing workers in one field may be unintelligible to those in another. A particularly vivid historical example of this situation is presented in the notion, once quite widely held, that the thermodynamic laws underlying life processes must differ in some essential way from those in force in the nonliving world—an idea whose untenability has only been generally recognized in rather recent years.

Differences of language and concept tend to be powerfully reinforced by many of the social factors governing scientific work. The desire of a scientist

to live and talk with those who will understand what he means, the pragmatic influences that inevitably make him seek professional identification with others in his immediate field, have their great strengths, both for the investigator and for his work. For the investigator, such association means immediate identification of interest and the satisfaction that only group activity can bring. For the research, it means the exposure of every man's work to intimate and continuing criticism by his peers in the same general subject area—the only critical estimate that can be truly meaningful or can really maintain the standards of the field. Yet there is a profound debit in this process too. At its worst it can harden an incipient conventionalism, and can raise the most serious barriers to communication within the body of science, powerfully reinforcing that separation of fields which, unchecked, leads to unbridled specialism with all its attendant ills.

These challenges to communication within the framework of science are severe enough. But today a further, and to some extent an intractable, threat of fragmentation is posed by the very magnitude of the scientific effort itself and by the tremendous volume of scientific publication that necessarily goes with it. This is a threat which has been increasing with immense rapidity over the half-century span of the Carnegie Institution. It is the worse because it is not only the sheer volume of paper, of titles, of content that must be dealt with. Some progress has been possible here through modern aids to storing and sorting information, and intensive research could doubtless carry their effectiveness much further.

But the hard core of the problem remains. It is the basic challenge to communication that lies in all the diversity of the natural sciences. It is the effective "addition" and the fruitful synthesis of ideas even in one field of work, and much more generally the transfer of idea-systems from one field into another, that, successfully met, may lead to major innovations of viewpoint.

Communication of this sort—the counterweight to the forces of fragmentation in science—can be greatly aided by environments of a very particular kind. There have been notable examples of them in every scientific age—in the great universities, and, more recently, in the great research institutes. They have comprised communities of investigators, working together in a common mode but in divergent fields, in continuous converse, in sympathy and in rivalry, without predetermined goal, without overcommitment as a body to any given sector of nature or to any one approach to the natural world. From such environments has come a goodly proportion of the real conceptual advances of science.

As the forces of fragmentation and diversity in science are clearly more powerful, the barriers to interchange evidently higher and more formidable, in our own day than in any other age, this kind of communication within science is more important now than it has ever been. It may, indeed, be one of



the most important aspects of the whole scientific effort if conceptual advance is to continue and to accelerate.

The creation of such an environment is a task to which the Carnegie Institution of Washington is dedicated, and for which it is unusually well equipped. The scientific community that is the Institution includes among its members almost the full range of gifts and attitudes that has been described. The scientific fields to which it addresses itself in the various Departments range from the primarily descriptive to the primarily analytical, from the pioneer to the more sophisticated. Yet by virtue of the mobility of its organization and its community of spirit, neither workers nor fields are isolated. Rather the reverse is true, so that fields of the most divergent character are sometimes included within the working frame of a single Department and even within the purview of a single investigator. These circumstances, and the fact that the whole of the Institution's work is pointed toward the end of uncommitted research, fit it peculiarly to assist in the major task of scientific synthesis.

The Institution has accomplished much in this direction, and its task in the future will be yet greater. Notably in the fields of astronomy and physics, and of physics, chemistry, geology, and biology, syntheses of concept and subject matter which have been and are being achieved contribute significantly not only to the breakdown of barriers between those fields but to the creation of new fields—fields that then lie open to be tilled.

Substantive work of this kind must always remain the most enduring basis for leadership by the Institution in this task. But there are other avenues too. Symposia, carefully considered, painstakingly organized, and sensitively timed, can be exceedingly fruitful in scientific synthesis and in the generation of new concepts, especially if they conjoin fields that are subtly related and bring together scientists from America and abroad who would normally foregather seldom if at all. A number of such symposia have been organized by various members of the Institution staff, and additional ones are contemplated.

The geographic dispersion of the several Departments of the Institution and the location of many of them near universities bring further opportunities to assume the role of a "crossroads" in the scientific effort, through the many kinds of informal working arrangements that are possible between the staff of the Institution and of these and other educational establishments. These potentialities have been considerably explored. They must be developed yet further.

Finally, the various fellowship programs, augmented now by the first of the Vannevar Bush Fellowships from the Massachusetts Institute of Technology, offer splendid opportunities to bring investigators to the Institution for varying periods of training, of collaborative work, or of independent creative activity. The newest, and one of the most exciting, of these programs has been designed to further the work of mature and senior investigators of distinction in the

various fields of Institution interest, both at home and abroad. Its initiation last year was made possible by a generous gift from the Carnegie Corporation of New York. Guests are currently expected from Holland, Denmark, and Great Britain, as well as from the United States, to be with the Institution for varying periods.

Such are some of the challenges to communication presented by the formidable diversities characteristic of the scientific effort. But there is a yet more important message which must be kept vivid if the promise in our scientific age is to be wholly realized. It is that of the deeper unities that underlie all the diversities of the scientific mode—the unities of value, of standard, of goal, of motivation.

To assume that human communication must always be in words, or even that it must always take place at the level of consciousness, is an undue restriction of viewpoint. Indeed, there is much to suggest that the kinds of communication which have had the most profound significance in human affairs have often been neither wholly conscious nor entirely verbal. They have come instead through the most powerful of all media—the sharing of a common experience or a common view, simply and grandly symbolized. The sun and the moon and the stellar firmament, that all men could see and equally know, must have provided such symbols to innumerable human groups far more remote in time than the great societies of Peru or Minoa or the Nile. J. Z. Young has drawn attention to the enormous power of the mountain or the hilltop, first natural, then man-made as the tumulus or mound or pyramid or temple, as a towering symbol of communication in ancient societies, and it is hard to conceive the whole structure and orientation of Renaissance Europe apart from the glory of its cathedrals, or to reconstruct that society in imagination without them.

If communication of the most profound sort can thus be nonverbal, it can also, of course, be largely divorced from material stimuli, a situation well illustrated by many highly evolved systems of religious belief. Few bonds of communication can have been stronger, for instance, or can have had a more important influence on the cohesiveness and the world view of a whole people, for good and for ill, than that Augustinian concept of knowledge and research that so dominated early Puritan America. As Perry Miller has described, its essence was that men must believe in order to know; that the conclusions of all possible investigations about the world are already given in advance of the search; that the most that right reasoning can possibly do is to arrive at them again by a parallel course and illuminate them in detail; that since reason is in any case fallible and likely to fall short of even this secondary goal, it may be wisest to forego reasoning altogether. No one can deny the power of such a concept as an instrument of communication, as an integrating and stabilizing



force—or, in some measure, as a powerful brake to action and to originality—in the society of its day.

But its very opposite, the approach to nature typified by the sweep of the scientific revolution of the sixteenth and seventeenth centuries—the deep-lying belief that all knowledge about the world is *not* given in advance of the investigation, that there still are new and profoundly beautiful and exciting regions of nature and of the relation between nature and the observer that are accessible to reason and experiment and have not yet been laid bare, that the effort to explore them may offer spiritual and emotional rewards comparable to the most exalting of experiences in other fields—this approach has long since proved itself a mode of communication at least equal in its sweep, more flexible and perhaps more viable, and, above all, conducive to positive action, to the growth of ideas, and to human joy. And so the whole orientation of scientific research can be considered in one sense a powerful symbol, as shining and as dominating in its way as the simpler symbols of the sun or moon, and in this context as much nonverbal, as much the sensed epitome of a shared, and accepted, and dedicated way of life.

The symbol itself is now some three centuries old. It has not dimmed in those three hundred years, but it has changed extraordinarily in form. From its very beginning, moreover, it has been in one sense a dual symbol, and this duality has become emphasized in recent years, especially in our own country. Neither profound philosophy nor practical experiment was new to the seventeenth century, nor, for that matter, to the Greeks. What gave the scientific revolution its novel character and power—what in fact represented the very genesis of science—was that for the first time these two strands were effectively entwined. Science was philosophy joined to practical experiment. And in the early conjunction there was an implicit realization that the concept must come first, that experiment must serve as the trap for lines of evidence already vaguely conjectured, that, in the suggestive simile of H. S. Harrison, experiment is experience sharpened to a point—less divining rod than digging stick.

Had scientific research not been so eminently successful in a practical sense, or were our pragmatic genius as a people less, it might not be so important to make sure, in our day, not only that the symbol of the scientific way stays bright, but that the strands do not become unwound. In the event, it remains essential to recall the distinction and the interdependence between the strands—science as a way of getting things done, and science as a way of life and a viewpoint of the world. The first component needs little further emphasis than its own extraordinary achievements already bring. But the second, and inherently the more basic, element does require constant reaffirmation among us that it may retain all the vitality and the allegiance and the comprehension that are essential to its vigor. The task of such reaffirmation is especially important not only because this is the more subtle as well as the more fundamental side of

science, and therefore less casually appreciated, but also because it is in constant danger of being overwhelmed by its lusty partner and so lost to view.

This is the task to which, above all, the Carnegie Institution is dedicated. It is the most essential duty of the Institution to communicate, in virtue of its own mode and its own being, the essential verities of the scientific way that are too easily forgotten. On one side lie the joy and the underlying human values of the road of the investigator, the compelling life challenge that is offered to the seeker after ideas about the natural world, whoever and wherever he may be. On the other lie the great unities of approach and of preparation that bind those dedicated to the scientific path: the requirements of verifiability; the discipline of parsimony; the emphasis on individual effort with its exacting demands of preparation and dedication, of originality and imagination, of the maintenance of style. These are not new parameters for the best in living. They are as old as civilized humanity. But the scientific mode offers one of the means by which those priceless elements, so often confused or threatened with destruction in a crowded world, can be assured their proper and their permanent place. No era which lacks them or to which they have been lost can be great, whatever may be its other assets. Our time has no more precious heritage than these qualities—and few tasks more essential than to defend and reaffirm them.

## THE YEAR'S WORK IN REVIEW

As always, selection of the year's researches for inclusion in this review must be in large measure arbitrary. It cannot imply that those described here are necessarily more or less important, more or less striking, more or less significant in the last analysis, than other programs going forward beside them which might have been included. They are simply to be considered as representative examples of the work carried on during the year in the various programs of the Institution. The reader who is interested in these programs in greater detail is referred to the fuller individual reviews of the various Departments that follow.

The Mount Wilson Observatory has pioneered for many years in the study of stellar magnetic fields. The first definite indication of them was obtained in the sun by Hale in 1908, using the then newly completed spectrograph of the 60-foot solar tower on the mountain. Hale's finding that many of the lines of the spectra of sunspots were split into components which had the characteristic polarization of a Zeeman pattern provided firm evidence of the existence of such fields. But subsequent search for a general magnetic field of the sun gave such erratic results that Hale himself was never satisfied with the conclusiveness of the work that followed.

Almost forty years later the problem was attacked at the Observatory in a somewhat different way. The considerable velocity of axial rotation attributed to nearly all the A-type stars suggested to Horace W. Babcock that these stars might show relatively large magnetic fields. Early in 1946 he made spectrographic observations of one such star, 78 Virginis, and discovered a general magnetic field of between one and two thousand gauss. Since that finding, some hundreds of stars have been under observation with the coude spectrograph of both the 100-inch and the 200-inch telescopes on Mount Wilson and Palomar. Thousands of measurements of stellar magnetic fields have been made, and these have been collected for publication during the present year. Eighty-four of the listed stars show definite evidences of magnetic fields, 55 are probably magnetic, and a further 55 show no indications of a coherent magnetic field.

In 1952, encouraged by these results, Harold D. Babcock and Horace W. Babcock returned to the older problem of the magnetism of the sun. With a solar magnetograph constructed to take advantage of improved gratings and recent advances in photoelectric techniques installed at the Hale Laboratory in Pasadena, evidence has been obtained of magnetic fields of the order of one gauss over large areas of the sun's surface. Since this finding, a second improved magnetograph has been completed for the 150-foot solar tower on Mount Wilson, and, starting with the end of this report year, a daily record of the distribution of the magnetic field of the sun's surface is being made. The



results should be of great interest, especially in view of the fact that evidence from many sources increasingly suggests that magnetic fields are of wide occurrence and probably play a much larger role in astronomical phenomena than has hitherto been supposed.

Problems of stellar evolution continue to occupy a central position in the research of the Observatories. One of the important and intriguing aspects of this field is the question of the evolution of the chemical elements in stellar systems. G. R. Burbidge and F. Hoyle, with Dr. W. A. Fowler and Dr. E. M. Burbidge, of the Kellogg Radiation Laboratory of the California Institute of Technology, have continued their work on the synthesis of elements in the stars. Eight nuclear processes are found to be necessary to account for the known abundances of the 327 isotopes recorded in the solar system. The greatest portion of the energy production of stars is due to the "burning" of hydrogen (in a nuclear sense), producing helium. When the reaction occurs in a mixture of hydrogen with other elements, it can result in the building of isotopes of carbon, nitrogen, oxygen, fluorine, neon, and sodium. In the second process, the nuclear burning of helium fuel produces  $C^{12}$ , and, by further  $\alpha$ -particle addition,  $O^{16}$ ,  $Ne^{20}$ , and perhaps  $Mg^{24}$ . In the third pattern, the  $\alpha$  process, through charged-particle interactions, builds the remaining four-structure nuclei  $Mg^{24}$ ,  $Si^{28}$ ,  $S^{32}$ ,  $A^{36}$ ,  $Ca^{40}$ , and probably  $Ca^{44}$  and  $Ti^{48}$ . The fourth pattern, the  $\epsilon$  process, builds the elements from vanadium through nickel, comprising the iron peak on the abundance curve. It takes place at very high temperatures and densities. The  $\alpha$  process and the  $\epsilon$  process are both thought to take place shortly before the explosion of a star as a supernova. The fifth process (the  $s$  process) is a slow neutron-capture chain, thought to occur in the interiors of red giant stars. The  $r$  process, a rapid neutron-capture chain, is believed to take place in supernovae, and to build uranium and thorium, together with a number of lighter isotopes. The seventh route of synthesis, the  $p$  process, is a proton-capture or photoneutron process which is also thought to occur in some supernovae. The eighth path of synthesis, the  $x$  process, is not as yet fully elaborated. It may be responsible for building deuterium, lithium, beryllium, and boron, elements that are unstable in hydrogen burning in stellar interiors. Work on the nature of the  $x$  process is continuing.

One of the most striking features of far outer space is the great clouds of gas that occupy it. The sources of these clouds may be various. Some of them are thought to represent the remains of great cosmic explosions, such as those of supernovae. Two programs have substantially increased our knowledge of these gaseous nebulae during the past year. In one of them Osterbrock has taken advantage of the fact that the relative intensity of two forbidden lines of O II near  $\lambda 3727$  varies markedly with density in the range of densities found in these objects. Observations of these lines have made possible direct deter-



minations of the densities (and of the masses) of the Crab Nebula, the nebula in Orion, and several planetary nebulae.

In the second program Münch and Wilson, using a multislit technique, have obtained high-dispersion spectrograms from which the detailed distribution of velocities through a large object such as the Orion nebula can be measured. Large and abrupt changes in velocity have been observed, suggesting shock-wave phenomena rather than a simple turbulence.

In the last report it was mentioned that the year had been an active one in the comparatively new field of the study of celestial objects as radio sources. These investigations have been continued in several directions in the current year, both at the Mount Wilson and Palomar Observatories and in the Department of Terrestrial Magnetism. Preliminary results were obtained in the Department last year in the measurement of intensity or flux of radio sources at frequencies below 30 mc. The greatly increased solar activity of the past year, which has interfered with the optical redshift measurements of the far distant galaxies, has also made the observing conditions for these radio sources difficult—so difficult that the preliminary measurements of 1955–1956 cannot, in all probability, be repeated in the 12 to 15 mc range until, perhaps, 1963–1965.

A review of intensity measurements on discrete sources in the available radio spectrum, however, has underlined the present inadequate and unsatisfactory data in this important area of radio astronomy. Accordingly, a program of flux measurements on a few of the intense radio sources over a wide band of the useful spectrum has become an important project in radio astronomy in the Department. Dipole antenna arrays accurately calibrated and with very precise record scalings have been employed, and measurements have been made of Cassiopeia A and Virgo A at frequencies below approximately 100 mc. Cygnus A and Taurus A are also objects of investigation. It is planned to continue dipole measurements to the limits of sensitivity—possibly several hundred megacycles. Thereafter it is planned to standardize other antennas of large aperture against dipoles at the transition frequency and to use scaled versions of the large antenna in subsequent operations. It may thus be possible to extend the range of absolute measurements to 1000 mc or more.

Measurements of radio emission within our own solar system have also continued actively. An antenna effective for a detailed examination of the emission of radio-frequency energy from the surface of the sun presents a number of special requirements, quite different from those demanded of antennas used for stellar work. Such an antenna array, specially designed for detailed examination of solar radio emission, has been built by the Department at the River Road site near Seneca, Maryland. Preliminary scans of the sun's face made with this equipment have revealed localized bright sources which move

across the disk as the sun rotates. Further work should reveal much of interest in the finer structure of solar radio emission.

It will be recalled that one of the earliest findings in the Department of Terrestrial Magnetism in the field of radio astronomy was that the planet Jupiter was a source of radio noise. Further investigation of this phenomenon has indicated that there may well be a single center of activity on the planet, with an approximately uniform rotational period. No visual observations of Jupiter report surface features having a corresponding rotational period, suggesting that the source of radio radiation lies below the cloud level of the planet and may well be associated with its actual surface.

Two further developments of note in radio astronomy are under way in the Department. To obtain a detailed knowledge of the nature of radio sources, optical identifications must be made. So far, only relatively few celestial objects have been so identified in a satisfactory way, and the available evidence suggests that many radio sources may be distant galaxies fainter than the eighteenth magnitude. To extend the list of optical identifications, precise position determinations for a large number of radio sources should be made. Antennas constructed for precision position measurements must incorporate very special features. Such antennas are under experimental design at present. At the same time, a beginning has been made this year in the actual construction of a radio telescope to be equipped with a 60-foot dish—a project that has been under consideration and study in the Institution since 1955.

It is a long way from considerations of distant galaxies and stars—their age, their evolution, their physical characteristics—to similar investigations within the confines of our own planet. In some respects, however, the qualities of our terrestrial environment have an even more vivid and immediate quality for the imagination. Both the Department of Terrestrial Magnetism and the Geophysical Laboratory are deeply concerned with the properties of the earth's crust. In the Department special attention has been focused for several years on a recognition and understanding of many large-scale physical processes operating over long periods of geological time which have resulted, among other things, in the formation of the continents and the ocean depths, mountain ranges and high plateaus. A *sine qua non* for such understanding is a comprehensive and quantitative description of the crust of the earth at the present day.

To this end, seismic and gravity studies continue to form an important program of the Department's work. It will be recalled that this program has in previous years included studies on the Colorado Plateau and, as reported last year, in Alaska. This year an even more ambitious seismic study has been undertaken in the Andean highlands, using as indicators the explosions normally set off in the operation of large open-pit copper mines in southern Peru



and northern Chile. This program has been undertaken by the Department in co-operation with the United States National Committee for the International Geophysical Year.

The problem of the age of the various rock deposits of the earth has engaged the attention of earth scientists since the beginning of geology. Despite widespread and intensive work over the years, however, major problems remain. Several years ago a program was undertaken jointly by the Geophysical Laboratory and the Department of Terrestrial Magnetism to extend the scope of mineral age measurements involving radiogenic products, originally limited to rocks containing uranium and thorium—an extension made possible by improvements in mass spectrometric techniques and finer developments of chemical analysis by isotope dilution. Particular emphasis has been given to the means made available by the natural radioactive decay of potassium to argon, and of rubidium to strontium.

Agreement of the rubidium-strontium and potassium-argon ages for a sample of mica is a good indication that the mineral was formed in a closed system, and such agreement is now considered essential to a satisfactory age determination. During the year the Geophysical Laboratory-Department of Terrestrial Magnetism group began an investigation of regional regularities in the ages of the Precambrian rock exposures. As an early result, it has been found that over a large part of Arizona, New Mexico, Colorado, and Wyoming rocks were formed during a period 1300 to 1400 million years ago. In Ontario there exists a large group of minerals that are apparently much older. Investigation of these, together with the work of other laboratories, suggests that there is a long band of rocks, extending from Wyoming through Montana, Minnesota, Manitoba, and Ontario into Quebec, composed of minerals of approximately 2600 million years of age. Similar areas of very ancient rocks are known in Africa and Australia.

A striking specific finding in the past year in this area of research has been the establishment, by concordant rubidium-strontium and potassium-argon datings, of an age of about 340 million years for the micas in a number of granitic rock samples collected by Dr. Faul, a guest investigator from the United States Geological Survey, from the Hercynian Chain of western Europe. Now the stratigraphic age of these rocks is known to be older than the middle Carboniferous, and they are usually assigned to the lower Carboniferous. According to the United States Geological Survey version of the Holmes time scale, the Carboniferous began about 265 million years ago. By this time scale, then, these Hercynian rocks according to the present findings belong in the middle Silurian. Thus there is a tremendous difference between the present age determinations and the commonly accepted stratigraphic assignment. The discrepancy is so great that it follows either that the time scale is not correct

or that the accepted stratigraphic assignment of the formation requires considerable revision.

Phase-equilibrium relations among the major mineral groups continue to be studied intensively at the Geophysical Laboratory and are making available a whole series of geological "thermometers" which may be applied to igneous and metamorphic rocks, supplying means for learning a great deal about the conditions under which such processes as mountain building occurred. Similar types of thermometers are being developed for ore minerals. Thus Kullerud and his associates have performed the basic laboratory work preliminary to field tests of an additional series of sulfide geological thermometers, including two based on the stability of pyrite and covellite and a third on the composition of pyrrhotite formed in the presence of pyrite. The ultimate accumulation of a number of such thermometers will make possible extensive cross checks and permit the reliable determination of temperatures prevailing during ore formation.

For a number of years the Geophysical Laboratory has carried forward a vigorous program in crystallography, which is becoming perhaps one of the most vital fields in all of science today. Through the use of neutron diffraction, nuclear and paramagnetic resonance, and X-ray diffraction the structural chemist is learning how the precise determination of molecular arrangement leads to real understanding of chemical reactivity. The nature of the chemical bond as manifested in covalent, metallic, ionic, or intermediate types is related closely to structure. Interestingly, work on the crystallography of alkali phosphates, continued this year jointly with Drs. J. W. Gryden and Helen M. Ondik, has demonstrated the first crystalline ultraphosphate on record.

One of the high points in research for the year in the Institution has been the work of Chayes in the Geophysical Laboratory. Using an optical analogue, he has produced diffraction patterns similar to the types obtained from the interaction of X rays on crystals. By making variations in the analogue he has been studying the kinds of patterns that might be obtained in various types of order-disorder in crystals. This method may well represent a major "break-through" in crystallographic research.

During the year Libby has continued his investigations of simple methods of absolute counting of  $\beta$  radioactivity at the Geophysical Laboratory. He has evaluated the role of back-scattered radiation with greater accuracy, and has discovered the important effect of surface roughness in the measurement of soft  $\beta$  rays. One extremely practical consequence of these findings is that they may now make possible the introduction, into the high school and college classroom and laboratory, of isotopes of real chemical interest, convenient life-time, and low enough specific activity to be completely safe, permitting student use, for example, of the radioactive forms of acetic acid, hydrochloric acid, sulfuric acid, and the calcium salts.



Yoder and Tilley have continued their studies in the production of various basalts, with particular attention to the possibility that they can be obtained naturally by differentiation of a parental magma. Laboratory determinations of the products formed when a variety of basalts are melted and quenched, as well as comparisons with minerals obtained from synthetic melts, show that the basalts must have been formed from different original magmas. Eugster has determined the effects of oxygen on the stability of the iron mica, annite, thus demonstrating the feasibility of investigating the important biotite micas.

The Carnegie Institution is deeply concerned with research in fundamental biology. In five of the seven Departments, investigations are under way in various aspects of the life sciences. They range from the organization of molecular units in life processes through questions of the structure and function of relatively elementary subcellular entities such as the pools for amino acid concentration and synthesis in single cells, of more grossly defined intracellular structures such as the chloroplasts of the cells of green plants, and of such exceedingly complicated and critical cellular organelles as the chromosomes, to the organization and functioning of cells themselves in the developing metazoan embryo, and finally to questions of speciation and experimental taxonomy, involving the organization and the interaction of many-celled plants in natural populations.

In the Geophysical Laboratory, Abelson has continued his pioneering work on the synthesis of amino acids from simpler components under conditions simulating those believed to have obtained on the earth in remote geological periods. Analysis of the available geologic evidence has led to the hypothesis that the principal constituents of the early terrestrial atmosphere were carbon monoxide, nitrogen, and lesser amounts of hydrogen, water, and carbon dioxide. In a series of laboratory experiments he has simulated the effects of solar radiation on such an atmosphere and the associated oceans and found that a considerable production of organic compounds results, including the amino acids glycine, alanine, and serine, as well as more complicated substances. These amino acids are important starting points in the building of proteins by living things.

One of the very interesting consequences of the analysis of probable conditions in the atmosphere and the oceans of the earth at the time when life may have originated is that carbon dioxide, probably fairly abundant in the early atmosphere, must have served as a kind of buffer in the oxidation-reduction system, guaranteeing that the atmosphere could never have been very reducing. It seems impossible to visualize that any great concentration of such substances as methane was present together with the carbon dioxide. This picture contrasts sharply with some of the earlier ones of the supposed conditions under which life-like systems might have originated on the earth.

For a number of years, the members of the biophysics group in the Department of Terrestrial Magnetism have been especially concerned in tracing the pathways of synthesis of proteins and nucleic acids in microorganisms, concentrating their attention especially upon two representative forms, a bacterium and a yeast. During the earlier years, attention was directed especially to the synthesis of relatively small molecules. Later the studies were shifted to investigations of the metabolic pools which are the precursors of the macromolecules. Special attention this year has been concentrated on the organization of structure within the cell in terms of some framework larger than a protein molecule.

Studies of the metabolic pools have revealed the extremely interesting point that they are susceptible to osmotic shock, and must therefore be held in some osmotically sensitive structure. Work conducted this year strongly suggests that when the osmotic pressure of the medium is suddenly reduced a flow of water occurs into the sensitive structures, together with a slow loss of solute from the cell. The stretching of the pool structures that results leads to an increase of permeability, allowing a faster rate of loss of the solute molecules. It was found in one set of experiments, for instance, that radioactive  $\text{SO}_4^-$  and  $\text{PO}_4^-$  ions can be taken up after such shock in an amount corresponding to about 5 per cent of the cell volume at the external concentrations of  $\text{SO}_4^-$  and  $\text{PO}_4^-$ —a situation that cannot occur in the absence of the shock. Stretching of the pool structures and loss of solute molecules finally lead to a new osmotic equilibrium in the cell and to the recovery of the normal pool function. These experiments are the first in which the properties of the pool as a definite, distensible intracellular structure have been clearly indicated, and they are correspondingly interesting.

Through the use of amino acid analogues the mechanism in the cell which selects amino acids for protein synthesis has been investigated by observing the “mistakes” that a cell can make in protein formation. Through collaboration with Dr. Georges N. Cohen, of the Institut Pasteur, it has been shown that selenomethionine, for example, can completely replace methionine and support exponential growth in a methionine-requiring mutant of the bacterium *Escherichia coli*. The experiment, together with related ones conducted at the Institut Pasteur, demonstrates that the amino acid composition of a protein can be altered by the presence of such amino acid analogues in the medium.

Perhaps the most dramatic finding of the biophysics group for the year has been the demonstration that, under appropriate conditions, rather large particles containing nucleic acids, proteins, and lipids can be made to form “spontaneously” from disintegrated cellular material. When cultures of cells of *E. coli* suspended in a glucose-mineral salts medium were brought to pH 8, centrifuged, resuspended, and broken by being forced through a small hole at high pressure, again centrifuged to remove cell fragments and any unbroken



cells, and the resulting supernatant decanted and once more centrifuged and diluted, a quite clear fluid remained. When to this fluid  $MgCl_2$  and  $MnCl_2$  were added (only the manganese being indispensable), the clear solution became cloudy and a few hours later was found to contain in considerable density particles which were nearly spherical and ranged in size from about 1 to 5 microns. Since the particles have a definite size and shape, are quite stable, and are found to contain several of the constituents of bacterial protoplasm, they have been named "protomorphs." Further studies of these remarkable objects are planned.

The Department of Plant Biology has long maintained a central concern with the nature of the photosynthetic pigment chlorophyll as it occurs in the living plant. The problem has been attacked by a number of routes. Attempts to isolate the photochemically active components of disintegrated chloroplasts made several years ago showed that reaggregation of the finely dispersed fragments restored activity. The size of the chloroplast fragments used at that time, however, was still too large to permit the isolation of the chlorophyll complex in pure form. Considerably more success has been achieved in recent years by the characterization and partial purification of the protochlorophyll complex from leaves grown in the dark. The chlorophyll-protein complex formed by illumination of etiolated leaf material, being soluble in water, is more suitable for certain chemical studies than the natural complex obtained from dark green plants. The complex in fully developed leaves contains far more chlorophyll per unit protein, however, so that it is important that it be studied also. As such complexes are generally water-insoluble, methods of investigation not based on chemical isolation seem more promising than attempts at direct chemical characterization.

One evident method for investigation is a study of the chlorophyll absorption spectrum. Such a study, concerned with the detailed shape of the red absorption band of chlorophyll *in vivo* and of the way that its shape may be altered by various procedures, is under way in the Department at present. The red band of chlorophyll in living material appears to consist of two components having a wavelength difference of about 10 millimicrons. These two components have not been separated chemically. Their presence, however, may be indicated by the shape of the derivative of the spectral absorbance curve—though another interpretation is also possible. The Department is particularly fitted to undertake an investigation of this kind by virtue of its derivative spectrophotometer, designed and built in past years by French and his group, and now in full operation.

Since the evidence for the chemical differences between native and extracted chlorophyll rests so largely on spectroscopic data, it is especially important to be sure that the methods for measuring the absorption spectra of pigments in

living cells are highly reliable. During the current year Latimer has shown that large errors can be introduced in determining the peak positions of the absorption bands of cell pigments by selective scattering of the light by the pigment molecules—a phenomenon which is sensitively dependent upon the wavelength of the incident light in the vicinity of a pigment absorption band. This critical work does not appear to invalidate the conclusion that natural chlorophyll is spectroscopically different from the extracted material, but it does raise grave questions of the validity of comparisons of the peak positions of chlorophyll in living cells spectroscopically measured in different laboratories, or even of different samples in the same apparatus. It also raises the important question whether the double peak of chlorophyll absorption observed by derivative spectrophotometry is due to two actual components, or whether one peak may be an optical artifact.

Attempts to isolate the naturally occurring protochlorophyll holochrome have been continued during the year by Smith, who explored additional methods of fractionating the leaf extracts. The earlier reported purification of about 75 per cent was not exceeded, but a promising new source of etiolated leaves has been found in the tropical starch-crop plant taro, which will be interesting for future work.

Another approach to the study of the natural chlorophyll-protein complex is to separate it into its protein and chlorophyll moieties and then to recombine them. Dr. Wolf Vishniac, of Yale University, has succeeded in this effort, and has demonstrated that the recombined “halves” of the complex can again show photochemical activity. During the year Vishniac spent some time at the Department, experimenting with and demonstrating his preparations. In the course of the work he found that purified chlorophyll *a* can be used in place of the alcoholic leaf extract with which his earlier experiments had been done, and which contained substances in addition to chlorophyll.

A more functional kind of investigation of the nature of chlorophyll and its relation to growth processes has been under way in the Department for some time. This is a study of the interrelated effects of light intensity and temperature on the growth of various species of algae, performed by growing the cells on an agar surface having a gradient of temperature from left to right and a gradient of light intensity from front to back. The growth pattern produced on such a plate was found to be very different in different species of algae. In last year's report the L-shaped pattern of *Chlorella pyrenoidosa* was mentioned. It has been found recently that the vertical arm of this pattern is accentuated by a narrow temperature range around 32° C in which growth is very poor at high light intensities. On the higher-temperature side of this sharply defined zone is an equally narrow range of luxuriant growth. At temperatures below 32° C at high light intensity the growth is moderately profuse for a few degrees, then weakens gradually toward lower temperatures.



It is believed that the narrow temperature range occupied by dark green cells on plates several days old is artificially sharpened by effects secondary to the direct influence of intensity and temperature on growth.

Yet another approach to the problem of the role of light in the life of the plant is a study of the orientation and behavior of freely moving unicellular algae in the presence of light of varying wavelengths and intensity. Such studies of phototaxis in the unicellular green algae have been continued at the Department this year, attention being focused especially on the form *Platymonas subcordiformis*. When this alga exhibited a fixed light response, the reaction could not be altered immediately by changing either the intensity of illumination or the absolute or partial pressure of carbon dioxide or oxygen in the medium. On the other hand, the ions  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  were found to be directly involved, and to be antagonistic in their effects,  $\text{Ca}^{++}$  causing a negative and  $\text{Mg}^{++}$  a positive phototaxis. A number of other ions that were tested produced no reaction. The theory that the driving power in the flagellates is an "adenosine triphosphate motor" has recently been suggested by Links, in Holland. Such a hypothesis implies that the mechanism supplying the muscular energy of animals is similar to that which innervates the flagellar apparatus of algae. From muscle research it is thought that  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  ions may be involved with antagonistic effects in the ATPase activity. Thus the antagonistic effect of  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  in these experiments in phototaxis is particularly suggestive.

Lynch and French last year reported the very interesting finding that the Hill activity can be restored in ether-extracted chloroplasts by  $\beta$ -carotene. This year the work has been continued by Dr. Max Milner, visiting the Department from Kansas State College, who used somewhat more quantitative techniques. The surprising effect has been confirmed, and it has been shown that the optimum concentration of  $\beta$ -carotene required for reactivation is approximately 100 times greater than that used in the earlier work. These and related experiments suggest that one or more fat-soluble factors may be involved in the reactivation of petroleum ether-extracted chloroplasts in addition to  $\beta$ -carotene. Further work is planned to determine the nature and the mode of action of these components.

Perhaps no aspect of the living cell presents so many facets for investigation or leads to consequences of wider general interest than the mechanisms through which heredity is mediated and determined. Concern with the processes of heredity must underlie biological work throughout the Institution, but, in both functional and structural aspects, it is the particular province of the Department of Genetics.

On the functional side, the remarkable findings of McClintock concerning systems of elements in the cell nucleus which control the action of genes in

maize have continued to open new vistas. Study has been concentrated on a system that controls gene actions at two known loci, not directly related to the *Ds-Ac* system earlier reported, though the genes affected are themselves ones which, in other cultures, have come under *Ds-Ac* control. Particular attention has been directed to determining the number of recognizable elements composing the system and to studying the transpositions and the changes in modes of expression that can occur.

Work has also continued in the analysis of a structural modification in maize chromosome 9, preliminary investigations of which were reported last year. The substance of the chromosome is distributed between two components, one member comprising the distal third of the short arm (the "fragment chromosome") and the other the proximal two-thirds of the short arm and all of the long arm (the "deficient chromosome"). The fragment chromosome exhibits aberrant behavior in somatic cells, sometimes being lost, sometimes undergoing changes in structural organization or becoming attached to ends or centromeres of other chromosomes, or being incorporated into another chromosome. The frequency of occurrence of events leading to such consequences and the time of their occurrence during the development of a tissue are both known to be under genetic control. Initial interest in this structurally modified chromosome was aroused by the aberrant behavior of fragments in somatic cells. It was later discovered that the fragment could behave unexpectedly in some of the meiotic cells also, and in plants either heterozygous or homozygous for the structural modification. It has become clear that the rules supposed to apply to crossing over in maize are not always followed by the fragment chromosome when it participates in a crossover event. The genetic significance of these findings may be very considerable indeed.

For a number of years Demerec and his group have been concerned with the application of methods of biochemical genetics to investigation of the detailed constitution of bacterial chromosomes—methods so quantitative as to be capable of resolving loci concerned with specific amino acid syntheses. In this connection they have paid much attention to the phenomena of transduction, in which fragments of a bacterial chromosome can be transferred from one bacterium to another through the vehicle of a particle of infective bacteriophage, and of transformation, in which genetic changes can be mediated by nucleic acid components of a cell. Such studies have been continued this year in the genetic mechanisms of the bacterium *Salmonella typhimurium*. Stocks have been constructed having various combinations of genetic markers representative of a cystine locus and four tryptophan loci so closely linked that they are carried together in one transducing fragment. Preliminary experiments with such stocks have indicated that three separate portions of a fragment may be incorporated simultaneously into a newly formed bacterial chromosome. If incorporation is accomplished through a process similar to crossing over in



higher organisms, six simultaneous crossovers would be required to produce the combinations of markers observed in these experiments. A most interesting indication has been obtained by Ozeki that a transducing fragment may not be a randomly selected section of a bacterial chromosome. The evidence suggests that the chromosomes of donor bacteria (infected with bacteriophage) are partitioned during the lytic process into small sections in some regular way and at predetermined locations, producing uniform transducing fragments for any one region.

New evidence that the "chromosome" of bacteriophage T2 consists exclusively of nucleic acid has been obtained by Hershey and his group. They have found that the chromatin material can multiply in functional form in bacterial cultures containing chloramphenicol, which inhibits the synthesis of all bacteriophage proteins.

The nature of reverse mutations in bacteriophage has been investigated during the year by Streisinger, with interesting results. Streisinger's previous work with bacterial viruses had revealed that mutations at many sites within a certain genetic region may result in a loss of the capacity of the bacteriophage to attach to certain strains of bacteria. Such mutations, resulting in a loss of adsorptive capacity in the phage T2, may take place at any of a large number of genetic sites. It is now clear that reverse mutations restoring this adsorptive capacity occur at precisely the sites of the forward mutations from which they are derived. Not all reverse mutants, however, are identical with the original, non-mutated form.

Work has also gone forward actively during the year on the structural side of the nature of the hereditary mechanism. Kaufmann, Gay, and their associates are combining methods of enzymatic hydrolysis with modern techniques of electron microscopy. Results obtained after deoxyribonuclease treatment of salivary-gland chromosomes of *Drosophila* fixed in osmium tetroxide and stained by the Feulgen method suggest that the main structural fibers are immune to nuclease or acid hydrolysis. DNA thus appears to be attached to the main chromosomal axis. Results obtained with chromosomes of *Drosophila* and of plants preserved in other fixatives are somewhat ambiguous; much work remains to be done before a generalized conclusion can be formulated about the location of DNA, RNA, and protein in chromosomes. Inclusion of tritium-labeled thymidine in the food of *Drosophila* larvae has afforded some spectacular radioautographs, prepared in collaboration with Philip Woods, of the salivary-gland chromosomes at late third instar. Radioactivity was limited to the banded regions. This finding suggests that thymidine, which is a precursor of DNA, was not incorporated into the material that occupies the intervals between the bands.

McDonald has continued her investigations of the intracellular deoxyribonucleases, which appear to be related to the general problem of DNA me-

tabolism. These deoxyribonucleases are particularly interesting because of the unique biological importance of their known substrates as components of chromosomes. McDonald has concentrated her efforts this year on a survey of the deoxyribonucleases of various tissues, seeking to determine their precise relation to cell division and to the synthesis and metabolism of DNA. During these studies it was found that the deoxyribonuclease content of salmon testes is especially high, and further investigation suggests that this tissue may be a potent and extremely useful source of the material.

The organization of cells into tissues, and their modification in the course of growth and metamorphosis of the many-celled animal, is the peculiar concern of the Department of Embryology. During the year some methods have been developed and applied which are most noteworthy in their delicacy and in the extent to which they permit exact inquiry. Perhaps one of the most striking is illustrated by the current work of Wilt, concerned with a system which may well permit study of the effects of a hormone system in a metamorphosing vertebrate at a molecular level. The bullfrog tadpole is transformed into an adult under the influence of the thyroid hormone. During the process, an abrupt change takes place in the visual pigment of the retina. The tadpole's retinal pigment, porphyropsin, is rapidly replaced by rhodopsin, the pigment of the adult retina. These visual pigments are composed of a protein moiety (opsin) coupled with a carotenoid. The change at metamorphosis has been described as a shift from retinene<sub>2</sub>, the aldehyde of vitamin A<sub>2</sub>, to retinene<sub>1</sub>, the aldehyde of vitamin A<sub>1</sub>. Wilt's long-range objective is to determine the role of the thyroid hormone in this shift. His immediate goal is to explore the metabolic implications of the predominance of retinene<sub>2</sub> in the tadpole and of retinene<sub>1</sub> in the adult. He first tested the hypothesis that the tadpole and adult differ in their content of oxidative enzymes, so that the tadpole can oxidize only vitamin A<sub>2</sub> and the adult only vitamin A<sub>1</sub>. His work disclosed that the two metamorphic stages do not differ qualitatively with respect to retinene reductase, the enzyme required for the reaction. Both the tadpole and the adult can oxidize vitamins A<sub>2</sub> and A<sub>1</sub>. In further experiments Wilt succeeded in preparing and characterizing highly purified porphyropsin and clearly showed that it differs from the rhodopsin of the adult, after which he used this porphyropsin in experiments of great interest. The question has been raised whether the pigment difference in the tadpole and adult was the result of a change in the ability of the opsin moiety to couple with the retinene. Thus the larval opsin might couple with retinene<sub>2</sub>, that of the adult only with retinene<sub>1</sub>. Wilt has been able to prove that larval opsin (bleached porphyropsin) will combine with retinene<sub>1</sub>, to regenerate rhodopsin, the kinetics of the reaction following closely those for the regeneration of rhodopsin in the adult retina. He is now



focusing attention on the pathways of synthesis of vitamin A<sub>2</sub> and on the mechanism that confines it to the eye.

In the course of experiments designed to modify the onset of synthesis and functional activity of acetylcholine in the early chick embryo, DeHaan made an unexpected observation that has led him to extend his study of spontaneous contractility to include an analysis of the morphogenetic movements of mesoderm and endoderm involved in cardiogenesis. It is often unappreciated that the factors regulating cell movements in the embryo include perhaps the most obscure phenomena in embryology. Thus it proved of great interest when DeHaan demonstrated that, in the chick embryo cultivated *in vitro*, the addition of a tiny crystal of acetylcholine to the surface of the endoderm resulted in the formation of two independently beating hearts. On the basis of several lines of evidence DeHaan has suggested that acetylcholine acts here by the sequestration of calcium or other divalent cations. Particular interest attached to the specificity of the effect. Other morphogenetic movements presumably are in progress at this time, yet only the cardiogenic movements are altered. DeHaan continues to probe this question, at the same time maintaining his interest in the onset of spontaneous contractility.

In consultation with Bishop, Dr. Katsh has continued to explore the unusually interesting observation that the injection into the male guinea pig of testicular extracts combined with adjuvant (a mixture of oils and certain killed bacteria) leads to the destruction of the spermatogenic elements in the testes of the recipient. It is suggested that damage to the spermatogenic tissue is the result of an immune reaction, which may operate in the following manner. The preparation of the testicular homogenate and its combination with adjuvant result in a change in its properties sufficient to enable it to act as an antigen in the homologous species. The antibodies produced by the host, however, are not sufficiently specific to distinguish normal and slightly altered testicular antigens; hence the animal's own testis is destroyed. Katsh's studies have disclosed that sperm-immobilizing, sperm-agglutinating, and complement-fixing antibodies appear in the sera of the injected animals and not in the sera of animals injected with adjuvant alone. Yet there are reasons for believing that these circulating antibodies may not be instrumental in inducing aspermatogenesis. For example, it has not been possible to demonstrate a correlation between antibody content and aspermatogenic effectiveness. Moreover, although other animals like the rabbit are highly effective in producing circulating antibodies of this type, aspermatogenesis can be induced only with difficulty. These considerations led to investigations of other types of immune reactions, as a result of which Katsh has found that the ileum of the sensitized guinea pig injected with homologous testis or sperm responds *in vitro* to a challenging dose of guinea-pig sperm by contracting, a finding that suggests an allergic response of the anaphylactoid type. While exploring this possibility, Katsh has made progress toward eluci-

dating the nature of the antigenic stimulus. He has demonstrated that the antigens are not species-specific, and that they are shared by brain and testis. Within the testis, the effective substances are confined to the spermatogenic elements, a fact established by the failure of homogenates of testes depleted of spermatogenic tissue to elicit a reaction. A number of thought-provoking experiments suggest, but do not prove, that the antigenic stimulus is provided by a mixture of bacterial and testicular lipopolysaccharides.

During the past years, Dr. Ramsey's morphological studies have led her to conclude that the circulation in the maternal placenta of primates is effected by the *vis a tergo* of the maternal blood pressure, a hypothesis that contradicts the traditional belief that the myometrial contractions "squeeze the placenta like a sponge." Although a few valuable observations have been made in pioneer studies on human patients, experiments are required to confirm or, if necessary, modify the hypothesis deduced from morphological studies. During the year, Ramsey initiated such a program in collaboration with Drs. G. W. Corner, Jr., and W. Newton Long, Jr., using experimental material from the Carnegie monkey colony. Although the first year of the research was devoted primarily to working out new techniques, the initial results have been encouraging. Standard procedures were devised for introducing a polyethylene catheter into the amniotic cavity—the uterus having been exposed at laparotomy—or into the intervillous space or a uterine vein. Positive results have indicated that myometrial contractions are reflected in heightened amniotic and intervillous space pressures, and that variations of absolute pressures in the amniotic and intervillous spaces correspond. Data have been obtained showing inherent amplitude-tonus patterns characteristic on the one hand of individual uteri and on the other of specific developmental periods in animals studied during a single pregnancy. Although the preliminary findings support Ramsey's hypothesis, the research is just beginning.

At the "highest" level of biological organization, the structure of populations, the group in experimental taxonomy is continuing its work actively at the Department of Plant Biology, though this year with a somewhat altered direction. The research has long been concerned especially with morphological and genetic studies of widely distributed groups of plants, directed primarily toward the clarification of the evolutionary relationships between races, species, and groups of species, and toward their fitness to their natural habitat. Until recently this work was done by comparing the responses of identical population samples of races and hybrids of plants in contrasting environments, by extensive genetic studies, and by examining chromosome behavior in hybrid and parental forms of species variously related. Many of these long-range studies have been completed, and emphasis is now shifting to a study of the comparative physiology of closely related but ecologically distinct races. Research is



being carried on at present with some of the species whose genetic constitution and evolutionary history have already been considered. Current work is especially concerned with rates of respiration and photosynthesis of contrasting climatic races of the monkey flower, *Mimulus cardinalis*, and with comparative growth and physiology in many races of the aquatic duckweeds, the Lemnaceae, and in various groups of the yarrow, *Achillea*.

Laboratory analyses and studies of data resulting from recent field work by the Department of Archaeology during the year revealed a number of new and significant facets of the Maya culture. Studies by Proskouriakoff have developed some extremely interesting information bearing on the date of the introduction of metal into Yucatan, suggesting that this event may have occurred later than has commonly been supposed. Thompson, in a study of effigy incense burners recovered from Mayapan, was able not only to identify a number of the gods or personages represented but in addition to show how considerably the religion of Mayapan was influenced by foreign ideologies. He also succeeded in identifying a number of glyphs representing diseases—a rather striking finding.

The Department of Archaeology has this year given particular attention to finishing the various studies in hand, preparatory to the completion of its work in 1958. Effort has been directed especially toward the production of preliminary reports on the field work and to broader studies that will lead to definitive statements covering the results of the more recent program of researches in Yucatan.

### *Losses . . .*

On June 30, 1957, Dr. George W. Morey retired from the Institution, bringing to a close an association of forty-five years with the Geophysical Laboratory. On the following day he joined the Geochemical and Petrology Branch of the United States Geological Survey to continue his work—a vivid demonstration of his own conviction that the work of true inquiry is never done.

When Dr. Morey joined the staff of the Geophysical Laboratory in 1912 that Department was but six years old. Its interest then was concentrated particularly in the study of mineral-forming processes in the interior of the earth. The new staff member expressed some misgivings about the value of the contributions a physical chemist could make to such a program. Events, of course, belied this uncertainty. His initial precise quantitative work on phase equilibria in silicate systems, in fact, provided the basis for most of his subsequent research. The label “systems containing water and carbon dioxide,” by which he describes his field of study, covers myriad activities. Intensive experimentation with silicates and other minerals under extreme conditions of temperature and pressure, approximating those prevailing deep in the interior of the earth, was the starting point for new insights into the processes of mineral formation,

ore deposition, and the chemistry of magmatic differentiation. It also led to a new theory of volcanic eruption based on the tremendous pressures developed when minerals crystallize from an aqueous magma.

Dr. Morey is noted for his skill and ingenuity in devising new apparatus and techniques. The "Morey bomb" was an important tool in the first production of synthetic quartz, which, in turn, led to most significant research in the development and manufacture of optical glass.

During both world wars, Dr. Morey left the Laboratory to put his theoretical and experimental work on the chemical constitution of glass to practical application in the service of his country. In the first war he manufactured strategic optical glass for the War Industries Board and was general manager of the Spencer Lens Company; in the second, he was a member of the optical instruments section of the National Defense Research Committee and, as manager of a division of the Corning Glass Works, he was responsible for the design, construction, and operation of the largest optical glass plant ever built. He invented a new family of glasses with high refractive index and wide dispersion especially adaptable for photographic lenses, which have been extensively used by the Army in aerial cameras. All these contributions to industry, technology, and military objectives were based on replacing empiricism with precise knowledge of scientific principles. Dr. Morey is the author of an American Chemical Society monograph, *The Properties of Glass*, now in its second edition, a treatise that stands alone in its field, and of some hundred scientific papers.

Dr. Milton L. Humason, who came to the Mount Wilson Observatory in 1917, retired on June 30, 1957. Beginning as a janitor at the Observatory, he soon became a night assistant. Here he showed such gifts of observation that in 1922 he was made a regular member of the Staff of Investigators. His most important contributions have been in the spectrographic study of very faint and distant objects. It was largely from the measurements of velocity furnished by his early spectrograms of distant galaxies that Edwin P. Hubble developed his famous hypothesis of an expanding universe. In 1928, at the request of Hubble, Humason began a long series of spectrographic observations of galaxies designed to investigate the relation between the redshift and the apparent brightness of the objects. These observations were continued over a period of twenty-eight years, with the aid of ever newer and more sensitive photographic plates and of modern spectrographs. After 1950, with the greater light-gathering power of the Hale telescope, he was able to extend the record of the spectra of galaxies to distances in space hitherto utterly unattainable. One of his particular achievements was the development of procedures for locating images accurately on the slit of the spectrograph and holding them there through long exposures. In the course of his extensive and precise observations he accumu-



lated redshift measurements of 620 separate galactic objects. For this work on redshift measurements he was awarded the degree of Ph.D. *honoris causa* by Lund University in Sweden in 1950.

From 1948 until his retirement Dr. Humason was Secretary of the Mount Wilson and Palomar Observatories, effectively handling many of the administrative tasks in addition to his scientific work.

One of the most painstaking investigators of the Institution's astronomical staff, Dr. Seth B. Nicholson, retired on June 30, 1957, after forty-two years of service. Dr. Nicholson came to the Institution from Lick Observatory, where he had already won recognition by the discovery of the ninth satellite of Jupiter in 1914. Subsequently he detected three other satellites of the planet—the tenth and eleventh in 1938 and the twelfth in 1951, all with the 100-inch Hooker telescope on Mount Wilson. Using the 48-inch schmidt camera on Mount Palomar, he has recently taken a series of photographs of the region around Jupiter, which he expects to use during the coming year to determine the relative brightnesses, positions, and magnitudes of all twelve satellites.

When he first came to Mount Wilson, Nicholson collaborated with the Director of the Observatory, Dr. George Ellery Hale, in investigations of the sun. Ever since, his work has been chiefly concerned with solar astronomy, more particularly with the phenomena of the visible surface of that star. He has carried out extensive studies of the magnetic polarity of sunspots.

Karl Ruppert, a staff member of the Department of Archaeology, retired on October 1, 1956. He joined the Institution group carrying on research in Middle American archaeology in 1925 and took an active part in the excavation and restoration of Maya buildings at Chichen Itza in Yucatan. Subsequently he made explorations in many other parts of the Yucatan Peninsula, some of which had never before been entered by archaeologists.

During World War II Ruppert took leave of absence, first for War Department activities connected with the American Legation at Guatemala City and later for ambulance work with the American Field Service in India, Burma, Italy, and Germany.

In the winter of 1947 he was in charge of a joint Carnegie Institution–United Fruit Company expedition to Bonampak, Chiapas, Mexico, the archaeological site that has become famous in recent years for its magnificent Maya mural paintings. Ruppert has described the location of the site, the history of its discovery, and its architecture in *Bonampak, Chiapas, Mexico*, written in collaboration with Thompson and Proskouriakoff.

In 1950 the Department of Archaeology began its survey of the ruins of Mayapan. In this project Ruppert, with A. L. Smith, conducted extensive excavations of the remains of dwellings, obtaining a rich collection of data the analysis of which should significantly add to our knowledge of the domestic

architecture, the patterns of settlement, and the size and character of the population of Mayapan and its vicinity.

Another member of the staff of the Department of Archaeology, Gustav Strömsvik, retired on June 30, 1957. Strömsvik was first employed in 1926 as a carpenter at Chichen Itza. He soon acquired such knowledge of that site and such skill in the engineering aspects of excavating, restoring, and protecting the ruins as to become one of the most valuable staff members in the field. In 1933 he carried out comprehensive repairs in the Temple of the Phalli at Chichen Itza, guaranteeing its preservation after excavation. From 1935 to 1942 he was in charge of the joint undertaking of the Government of Honduras and the Carnegie Institution to excavate, restore, and preserve the highly significant ruins at Copan. Here he re-erected the extraordinary monuments of that city, repaired several of its most important buildings, and restored the great hieroglyphic stairway. In the course of this program it became necessary to divert the course of the Copan River to prevent its violent summer floods from undermining the ruins—a major engineering undertaking.

Strömsvik enlisted in the Royal Norwegian Navy in 1943 and subsequently took part in the landing of Allied forces on the Normandy beachhead. When he returned to the Institution in 1945 he resumed his diverse activities of exploring, mapping, restoring ruins, and collecting artifacts. He participated in discussions in Honduras leading to the establishment of the Instituto Nacional de Antropología e Historia there, for which he served as a Technical Adviser. He has now returned to Norway, where he plans to continue his archaeological investigations.

For the past twenty years the Carnegie Institution has been particularly fortunate in having as its editor Miss Dorothy Swift, who retired on June 30, 1957. The importance of a gifted editor in any publications program cannot be overemphasized. Miss Swift's contributions to lucidity and exact expression won the respect and admiration of the staff. Her high standards and her meticulous attention to perfection in detail are reflected in the publications of the Institution during the years of her service. Miss Swift plans to continue her editorial work in Boston.

The death of Dr. John von Neumann, brilliant mathematician and member of the Atomic Energy Commission, on February 8, 1957, cut short an association which the Institution was deeply privileged to enjoy. He had been appointed a Research Associate on June 1, 1955.

Dr. von Neumann was born in Budapest and received his early training there and at Zurich. He came to the United States in 1929 as a visiting lecturer at Princeton University, where the following year he was made professor of



mathematical physics. Three years later he received appointment as one of the first full professors at the Institute for Advanced Study.

There were few fields of physics or mechanics untouched by von Neumann's genius. His pioneering investigations of the phenomena of weather are well known. Perhaps the impact of his gifts was most widely felt in the field of military armament. His discovery and development of the implosion method were critical to the development of the first atomic bomb. He was one of the principal advisers to the United States Air Force and a powerful exponent of the intercontinental ballistic missile. Von Neumann's contributions to the design of early fusion weapons were equally significant. He was the recipient of many honors, among them the Medal of Freedom and the Enrico Fermi award for his work on the theory and design of computing machines. In October 1954, he was appointed to the Atomic Energy Commission and served actively with it, in spite of his illness, almost to the time of his death.

Few losses to contemporary science have been as great as this premature and tragic ending of the career of a man of extraordinary genius.

On May 14 of this year Dr. Francis G. Benedict, formerly Director of the Institution's Nutrition Laboratory, died at the age of 86. He began his researches in human nutrition at Wesleyan University under grants of the Carnegie Institution. In 1907 he became Director of the Institution's Nutrition Laboratory in Boston and served in this capacity until his retirement in 1937.

The activities of the Laboratory during Dr. Benedict's thirty years as Director were concentrated largely on establishing standards of basal metabolism of normal human subjects according to height, weight, age, sex, and race. Special researches were made on the conditions that may affect basal metabolism, such as position of the body, temperature, vegetarian diet, athletic activity, environment, season, and fatigue. Notable contributions were made to the invention and testing of various types of apparatus for measuring heat production, heat elimination, respiratory exchange, and surface and internal body temperature.

Henry Norris Russell, who died on February 18, 1957, in his eightieth year, had long been one of the leading astronomers of this country. A brilliant scientist with an encyclopedic knowledge of astrophysics, a man of inexhaustible enthusiasms, varied interests, and boundless energy, he exercised an inspiring influence on all with whom he came in contact.

Although his career was centered in Princeton, he had associations with the Institution from its earliest days, first as a Research Assistant and later, from 1921 to 1945, as a Research Associate. He worked in England at the Cambridge Observatory from 1903 to 1905. In 1911, at the early age of thirty-four, he became director of the observatory at Princeton. From 1921 until the late



1940's the Institution had the benefit of his presence for a part of practically every academic year for research and lectures at Mount Wilson.

Dr. Russell's early investigations in the field of stellar constitution and evolution are of major significance. The Hertzsprung-Russell diagram of the relation between absolute magnitude and spectral type of stars has played a most important part in the advance of astrophysics. During the first World War he contributed to military research in the field of airplane navigation. He was a pioneer in the analysis of complex laboratory spectra. The role of spectra in the interpretation of the physical characteristics of stars absorbed him for several years. His discovery that hydrogen was by far the most abundant element in the atmosphere of the sun ran contrary to the accepted belief of the day. It is now a basic fact of cosmology.

Dr. Arthur S. King died on April 25, 1957, at the age of 81. He was one of the early Research Assistants of the Institution, receiving a grant in 1904 to investigate emission spectra at high temperatures at the Universities of Bonn and Berlin. On January 1, 1908, after teaching physics at the University of California for three years, he was appointed Superintendent of the new Physical Laboratory of the Mount Wilson Observatory, which was equipped with a 30-foot spectrograph and a large electric furnace designed by him, apparatus superior to anything then in existence for spectroscopic work.

Over the years until his retirement on February 1, 1943, Dr. King concerned himself with laboratory investigations of many spectra. His study of the temperature classification of the spectra of chemical elements, as observed in the arc, spark, and electric furnace, had a far-reaching effect, both on the analyses of complex spectra and on the interpretation of the solar spectrum, the sunspot spectrum, and stellar spectra in general. His research also included the study of the spectra of several of the rare earths (atomic numbers 57 to 71), and he completed the wavelength measurements and intensity estimates for a great number of lines in these complex spectra. His laboratory observations, in collaboration with those of Raymond T. Birge, led to the extremely important discovery of the carbon isotope of mass 13.

During World War II, Dr. King investigated the velocities of aerial torpedoes for the Office of Scientific Research and Development at the California Institute of Technology, and from 1946 to 1954 he served as a mathematician with the Naval Ordnance Test Station in Pasadena.

#### *. . . and Gains*

In consequence of a generous gift from the Carnegie Corporation of New York, the Institution is initiating this year a series of special fellowships in the natural sciences, primarily to permit of travel and visits of distinguished scholars to departments of the Institution carrying on research in their fields of interest.

Such awards are entirely without restriction with regard to their specific use, but are made in general in subject fields to which the Institution is especially directing its attention.

Current recipients of the new fellowships are Dr. Evelyn M. Witkin, of the College of Medicine, State University of New York; Dr. William A. Arnold, of the Oak Ridge National Laboratory, Oak Ridge, Tennessee; Dr. R. v. d. R. Woolley, the Astronomer Royal of Great Britain; and Professor Jan Hendrick Oort, the Director of the Observatory of Leyden in the Netherlands.

It is a particular pleasure to report that Dr. Vannevar Bush, retired President of the Institution, was elected Chairman of the Corporation of the Massachusetts Institute of Technology on March 4, 1957. On May 21, the New Jersey Patent Law Association in Newark presented him with the 1957 Jefferson medal for his notable contributions to the United States patent system.

It gives me great pleasure to announce the following honors that have been received by directors and members of the staff.

The Catherine Wolfe Bruce gold medal for 1957 for distinguished services to astronomy was awarded to Dr. Ira S. Bowen, Director of the Mount Wilson and Palomar Observatories, by the Astronomical Society of the Pacific. Dr. Jesse L. Greenstein, staff member of the Observatories, was elected to the National Academy of Sciences. Dr. Milton L. Humason was elected an associate of the Royal Astronomical Society. Dr. Harold D. Babcock, retired staff member, received the degree of Doctor of Laws *honoris causa* from the University of California.

At the Department of Plant Biology, Dr. Jens Clausen, retired staff member, was awarded the degree of Doctor of Agronomy *honoris causa* by the Royal College at Upsala, Sweden, on May 31, 1957, in connection with a celebration of the 250th anniversary of the birth of Linnaeus.

Dr. M. Demerec, Director of the Department of Genetics, was elected a member of the Royal Danish Academy of Sciences and Letters on April 6, 1956, and on February 3, 1957, he received the degree of Doctor of Laws *honoris causa* from Hofstra College, Hempstead (Long Island, New York).

The Geophysical Institute of Huancayo, Peru, has been renamed in honor of the late Dr. John A. Fleming, long associated with the Department of Terrestrial Magnetism, as the "Geophysical Observatory John A. Fleming."

Caryl P. Haskins





# REPORTS OF DEPARTMENTS

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## *and SPECIAL STUDIES*

MOUNT WILSON AND PALOMAR OBSERVATORIES

COMMITTEE ON IMAGE TUBES FOR TELESCOPES

DEPARTMENT OF TERRESTRIAL MAGNETISM

GEOPHYSICAL LABORATORY

DEPARTMENT OF PLANT BIOLOGY

DEPARTMENT OF EMBRYOLOGY

DEPARTMENT OF GENETICS

DEPARTMENT OF ARCHAEOLOGY



# MOUNT WILSON & PALOMAR OBSERVATORIES

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*Operated by the Carnegie Institution of Washington  
and the California Institute of Technology*

*Pasadena, California*

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## INTRODUCTION

In 1908, Hale, using the newly completed spectrograph of the 60-foot solar tower on Mount Wilson, noticed that many of the lines of the spectra of sunspots were split into components which had the characteristic polarization of a Zeeman pattern. This observation provided the first definite evidence for the presence of a magnetic field in an astronomical body. Later Hale and his collaborators searched for a general magnetic field of the sun, but the field was so weak that Hale was never satisfied with the conclusiveness of the evidence.

The large velocity of axial rotation attributed to nearly all A-type stars suggested to Horace W. Babcock that relatively large general magnetic fields might exist in these objects. Early in 1946 he made the necessary spectroscopic observations of one of these stars, 78 Virginis, with the 100-inch coudé spectrograph, and discovered a general magnetic field of between 1000 and 2000 gauss. Since that time some hundreds of stars have been under observation with the coudé spectrographs of both the 100-inch and the 200-inch. A few score of the most interesting objects have been observed many times in order to study the fluctuations that occur in the magnetic fields. Many of the variations are irregular, but in some stars the field reverses with a regular period of a few days. During the present year Babcock has collected for publication all the thousands of measurements of stellar magnetic fields made in the past 11 years. Eighty-four of the stars listed show definite evidence for a magnetic field; 55 are probably magnetic; another 55 show no evidence for a coherent magnetic field. One hundred and eleven of the stars observed had lines too broad for magnetic measurements.

Encouraged by the discovery of these general fields of rapidly rotating stars, Harold D. Babcock and Horace W. Babcock returned to the problem of the general magnetic field of the sun in 1952.

Taking advantage of improved gratings and of current advances in photoelectric and electronic techniques, they constructed a solar magnetograph which scans the surface of the sun and records the amount of the residual polarization at the side of a certain sensitive spectral line caused by the Zeeman splitting of the line in the magnetic field. Installed in the Hale Solar Laboratory in Pasadena, the magnetograph immediately gave definite evidence for magnetic fields of the order of 1 gauss over large areas of the solar surface. Furthermore, the pattern of many of these fields was found to change in the course of a few days; this finding explains why the results obtained by Hale and the other early observers were so erratic and inconclusive. Recently a second, improved magnetograph has been completed for the 150-foot solar tower on Mount Wilson. Starting at the end of the report year, a daily record of the distribution of the magnetic fields over the sun's surface is being made as a regular part of the solar program.

Meanwhile indirect evidence has been found at other observatories for small interstellar magnetic fields extending throughout large volumes of space. In particular the discovery in the USSR, by Dombrovsky and by Vashakidze, of polarization in the Crab Nebula led to the explanation that the continuous radiation from this object was synchrotron radiation caused by very-high-velocity electrons being accelerated in a magnetic field. Baade with the aid of the 200-inch was able to study the detailed structures of the polarization in the Crab Nebula and also to observe polarization in the radiation of the jet in M 87 which probably has a similar cause.

All these observations point to the fact that magnetic fields are very widespread and play a much larger role in astronomical phenomena than has hitherto been supposed. They also mean that any attempt

to explain the structure of stars and nebulae must more and more take into account the forces exerted on the ionized gas or "plasma" associated with these fields. Because of the growing importance of magnetic phenomena in astronomy a Symposium on Electromagnetic Phenomena in

Cosmical Physics was held in Stockholm on August 27-31, 1956, under the auspices of the International Astronomical Union. Three representatives from the Mount Wilson and Palomar Observatories attended, including H. W. Babcock, who was one of the organizers of the Symposium.

## OBSERVING CONDITIONS

For the fifth consecutive year, precipitation was below normal on Mount Wilson, the total for the year being 25.47 inches. Nevertheless, the number of cloudy days and nights was large, especially in the sec-

ond half of the year. Solar observations were made on 323 days; observations were made with the 100-inch on 292 nights, and with the 60-inch on 285 nights.

## SOLAR RESEARCH

### *Solar Photography*

Solar observations were made by Nicholson, Hickox, and Cragg. The numbers of photographs of various kinds taken between July 1, 1956, and June 30, 1957, were as follows:

Direct photographs . . . . .	638
H $\alpha$ spectroheliograms, 60-foot focus . .	567
H $\alpha$ spectroheliograms, 18-foot focus . .	936
K2 spectroheliograms, 18-foot focus . .	921
K2 spectroheliograms, 7-foot focus . . .	78,000
K prominences, 18-foot focus . . . . .	942

### *Sunspot Activity*

The magnetic classification and study of sunspots and related phenomena have been continued by Nicholson and Cragg. Co-operative programs have been carried out with the U. S. Naval Observatory, the University of Michigan, the Observatory of Kodaikanal, the Meudon Observatory, and the Central Radio Propagation Laboratory of the National Bureau of Standards. During the calendar year 1956, solar observations were made on 348 days, on none of which was the sun without spots. Only in 1953 were observations made on more days than in 1956. The total number of spot groups observed in 1956 was 642, compared with 208 in 1955 and 46 in 1954. The record number of groups ob-

served here in one year is 663 in 1947. The northern hemisphere was again the more active, with 53 per cent of the total groups.

An unusual number of spots have appeared in very high latitudes in this cycle. In the 65 years from 1878 to 1943, during which time daily solar photographs are available, only four groups that lasted longer than a day appeared on the sun farther than 40° from the equator. In the last cycle, 1943 to 1954, eight such groups were observed. In the present cycle thirteen have already appeared.

The rate of increase of solar activity from 1955 to 1956 greatly exceeded that from 1945 to 1946. If the number of spots continues to increase for another year, as it did at a similar phase last cycle, the coming sunspot maximum will certainly be the highest ever recorded. Since maximum activity is expected in 1957 a new record could be set.

The monthly means of the number of groups observed daily for the past two and one-half years are shown in table 1.

### *Magnetic Polarities*

Magnetic polarities in each spot group have, if possible, been observed at least once. The classification of groups observed between July 1, 1956, and June 30, 1957, is indicated in table 2. "Regular" groups in



the northern hemisphere are those in which the preceding members have N (north-seeking) polarity; in the southern hemisphere the polarities are reversed.

TABLE 1

Month	Daily Number of Sunspot Groups		
	1955	1956	1957
January .....	2.2	6.4	12.7
February .....	2.2	9.3	10.9
March .....	0.6	10.6	13.6
April .....	1.4	10.8	14.0
May .....	2.3	9.9	12.7
June .....	2.4	9.3	15.1
July .....	3.2	9.3	...
August .....	3.3	13.5	...
September .....	4.9	15.2	...
October .....	6.1	12.9	...
November .....	7.3	11.8	...
December .....	6.9	13.8	...
Yearly mean ....	3.6	10.2	...

TABLE 2

Hemisphere	Regular	Irregular	Unclassified
North .....	259	7	80
South .....	312	5	96
Whole sun. .	571	12	176

### *Studies of Geomagnetic Activity*

The study of the diurnal variation of geomagnetic activity on very disturbed days, reported last year, has been extended by Nicholson and Dr. Oliver R. Wulf, of the U. S. Weather Bureau, to include the ten selected quiet days in each month and the all-except-quiet days. The local-time component for the quiet days, which is quite significant, seems to be related to deviations from the normal diurnal change in the intensity of the earth's field. This effect, which changes the phase of the local-time component for all days, has been removed from the data. The remaining local-time component of disturbance reflects the known local-time dependence of

magnetic "bays." The additional Universal-Time component indicates that throughout the year disturbance is greater when the sun is over certain longitudes, following a pattern like that near the equinoxes, but that the variations are influenced by the north-and-south position of the sun as well. This effect may arise from the changing position of the ionization cap under the sun relative to the earth's magnetic field and to the changing patterns of the large-scale atmospheric circulation.

### *Solar Magnetic Fields*

In the past year, 160 magnetograms of the sun have been made by Harold D. Babcock; a total of 786 has been obtained since the series was begun in 1952. Compilation of additional data, not photographically recorded, up to the end of 1956, confirms published results on signs and intensities of the high-latitude polar fields of the sun.

Measurements have been made on weak coherent fields of great area for 17 dates between December 24, 1953, and March 14, 1954. For 29 of these fields the following mean values are found: maximum intensity, 1 gauss; total magnetic flux,  $10^{21}$  maxwells; areas, 0.14 of the sun's disk.

Measurements of magnetic flux, maximum intensity, and area have been made for fields of each sign in both sunspot zones for 21 days between October 8, 1955, and May 13, 1956. This interval includes the most notable outburst of activity in the first 18 months of the present solar cycle.

In 22 selected bipolar magnetic regions, each dominated by one sunspot, the flux, maximum intensity, and area have been measured. In each case, the maximum flux of the spot umbra has been estimated, for a comparison between the flux emerging from the spot and the flux returning through a photospheric region of opposite polarity.

A comparison of magnetograms with charts of coronal activity issued by the High Altitude Observatory, Boulder, Colo-

rado, based on emission of  $\lambda\lambda 5303, 6374$ , shows a general correspondence between magnetic flux and light flux. In every case, the areas on the sun are much more extensive in longitude than in latitude.

After putting the new solar magneto-

graph at the 150-foot solar tower on Mount Wilson into regular operation at a high level of stability and sensitivity, Vrabec has used it to map magnetic fields in the neighborhood of sunspots and to study their variations.

## PLANETS AND SATELLITES

### *Observations of Mars*

Mars was observed at the opposition of 1956 by Richardson on 34 nights for a total of 110 hours. About twice as much time was spent on the planet as in 1954, under generally superior conditions. Only part of the material secured has been reduced as yet.

Four hundred and ten direct photographs of Mars were taken in blue, orange, red, and infrared light with a camera and enlarging lens at the Cassegrain focus of the 60-inch. The diameter of the images ranged from 2 to 10 mm. Eastman IV F emulsion was used throughout the visual region, and IV N emulsion in the infrared. Exposure times ranged from  $\frac{1}{3}$  to 20 seconds.

The direct photographs, recording the appearance of the planet at the time of observation, can be used for photometry of the disk. From measures on the positions of the markings the oblateness of the planet can be determined, since the period of rotation is accurately known.

The effective wavelength of the red images is at 6600 Å, at about the position of the chlorophyll absorption band. The effective wavelength of the infrared images is at 8000 Å, in the region where chlorophyll reflects strongly. Photographs taken a few minutes apart show the intensity of the maria relative to the deserts as the same in both red and infrared. The maria, therefore, cannot consist of vegetation with a high chlorophyll content, unless the reflectivity of chlorophyll on Mars differs markedly from that of terrestrial vegetation.

Spectra of Mars were taken by Richardson on 17 nights with the 114-inch camera,

using the grating which gives a dispersion in the first order of 5.6 Å/mm.

Plates in the first order infrared were taken in a search for the carbon dioxide bands at  $\lambda 7820$  and  $\lambda 8688$ , first discovered in the spectrum of Venus. Carbon dioxide has already been identified in the Martian atmosphere through the band at 1.6 microns. The bands at  $\lambda 7820$  and  $\lambda 8688$  do not show in the telluric spectrum with a very low sun. There was no trace of these bands in the spectrum of Mars. Their absence should allow us to set an upper limit to the quantity of carbon dioxide in the atmosphere of Mars.

The A band of oxygen at  $\lambda 7600$  was photographed in the spectrum of Mars in July and December 1956, when the velocity of the planet relative to the earth was  $-8.9$  and  $+14.9$  km/sec, respectively. This velocity gives a shift of 0.62 Å, corresponding to 0.11 mm on the plates. Previous measures to detect oxygen have been made on the (1,0) B band at  $\lambda 6900$ . The (0,0) A band would seem better suited to this investigation, owing to the greater intensity of possible Martian components. Also, the isotope lines in the band of  $\text{O}^{16}\text{O}^{18}$  arise from molecules about 0.004 as abundant as those producing the principal lines, thus affording a basis of comparison for the intensity of Martian lines.

The presence of water vapor might be detected from variations in intensity of the  $\text{H}_2\text{O}$  lines in the maria and polar cap relative to the adjacent deserts. Inspection of the water-vapor lines on several spectra in the region of  $\lambda 8200$  showed no variations in intensity that seemed significant.

Direct photographs of Mars in visual light show the surface markings distinctly.



Those in blue and violet usually show only a blank disk with a greatly enlarged polar cap.

Preliminary measures by Richardson on the spectra from 7500 to 8500 Å give a ratio maria/desert  $\approx 0.78$ . No spectrograms were taken in the red, but the direct photographs indicate that this ratio remains nearly constant from 6000 to 7500 Å. Beginning about  $\lambda 5700$  the ratio maria/desert  $\approx 0.84$ , and increases gradually until at about  $\lambda 4400$  the maria and deserts become indistinguishable.

The polar cap is inconspicuous in the infrared, increases steadily in intensity in the visual region relative to the deserts, and is apparently still increasing at the limit of our spectra at  $\lambda 3600$ .

#### *Spectra of Venus*

Thirteen spectra of Venus were taken by Richardson for the measurement of rotation in the  $\lambda 6300$  region with the 114-inch camera in the second order with a dispersion of 2.8 Å/mm. Two spectra of Mars were taken as controls. Fifteen spectra of Venus have also been taken at the Snow telescope with a dispersion of 0.75 Å/mm. Only a few exploratory measures have been made on these plates.

#### *Satellites*

Perturbations in the motions of the satellites furnish one of the best means of

determining the oblateness and inner constitution of Mars. A dozen of Richardson's best plates which show both Phobos and Deimos have been sent to the U. S. Naval Observatory at their request for measurement and reduction.

Photographs of the satellite field around Jupiter were taken by Nicholson with the 48-inch schmidt on several nights in April, May, and June with corresponding exposures on Selected Areas to redetermine the magnitudes of the faint satellites in a homogeneous manner. Positions of the satellites will also be measured on these plates, each of which covers the entire satellite field, and their search should reveal all of Jupiter's satellites down to the limiting magnitude of the plates. The search, measurement, and reduction of these plates, the last of which were taken in June, have just been started. A preliminary search of the plates taken in April located all the known distant satellites with JXII near the plate limit. No new satellites were found, but some fifty asteroids were detected in the search.

Two pairs of plates of Saturn taken in June by Osterbrock with the 48-inch schmidt have been searched for satellites by Nicholson. No new satellites were found. Among the asteroids detected, one has the motion characteristic of the Trojan asteroids, but further observations will be necessary to determine its orbit.

### COMETS

Reduction was completed, under the supervision of Osterbrock, of a two-year series of Comet Baade (1954h) and Comet Haro-Chavira (1954k). These comets were at a distance of approximately 4 astronomical units from the sun during the whole period of observation, and the plates were taken with the 48-inch schmidt in order to study the comets' tails at these great distances. The position angle of the projection of the comet tail on the sky was measured on each plate; the reductions show that this angle is not at all strongly correlated with the position angle of the

projection of the line from the sun to the comet, but is strongly correlated with the position angle of the projection of the comet orbit behind the comet. The reductions further show that, on the reasonable assumption that the comet tail is in the orbital plane of the comet, it lies approximately halfway between the line from the sun through the comet and the line along the orbit behind the comet. This indicates that, unlike most of the well known bright comets observed close to the sun, in which radiation pressure dominates the direction of the tail, in these more distant comets



the pressure of the interplanetary matter is roughly as important as radiation pressure in its effects on the tail. As the density of interplanetary material is not believed to be particularly high at great distances from the sun, presumably the material in the

tails of these distant comets is relatively ineffective in absorbing solar radiation.

An extensive series of photographs of comet Arend-Roland (1956h) were taken with the 48-inch schmidt camera by Nicholson, Minkowski, and Schmidt.

## STELLAR SPECTROSCOPY AND PHOTOMETRY

Spectroscopic observations have been regularly carried out with all major telescopes except the 48-inch schmidt during the moonlit half of each month. Seven hundred spectrograms were made with the 200-inch telescope, 827 with the 100-inch, and 978 with the 60-inch during the year.

### *White Dwarfs and Subdwarfs*

The survey of white-dwarf spectra by Greenstein now includes about 60 objects. Hydrogen-line profiles were obtained for 25 normal objects of type DA. The use of the prime-focus spectrograph of the Hale telescope permits spectra, well widened, to be obtained at the dispersion of 160 Å/mm down to 15.5 mag. Fainter stars seem difficult during the present sunspot maximum because of the increased brightness of the airglow, whose emission lines are now easily detectable on exposures longer than an hour. Various groups of peculiar objects have been discovered. The star HZ 29 has extremely shallow lines of He I. The star HZ 21 seems to be one of the hottest probable white dwarfs, of spectral type dO. It shows very strong lines of He I and He II. A new type of yellowish white dwarf, represented by W 219 and L 879-14, has been found to show an unidentified broad absorption near  $\lambda 4670$ . A recurrent nova, WZ Sagittae (1913, 1946), has broad hydrogen absorption lines similar to those in a normal white dwarf underlying its broad emission bands. Its luminosity derived from proper motion is near +10, so that it seems to be the expected link between the white dwarf and the nova stage of evolution.

Most old novae so far observed have relatively narrow emission lines, presumably

arising from post-nova ejection of matter from the star. Their blue continuous spectrum shows no distinct sharp or broad absorption lines. Nova DQ Herculis has relatively broad emission lines, with the higher members of the Balmer series variable through the 4-hour cycle. A first theoretical analysis of the observations of white-dwarf spectra has been made by Greenstein with the goal of obtaining a temperature scale largely based on colors and the central depth of the hydrogen line. The colors should be very reliable for the white dwarfs with nearly continuous spectra. On the assumption that the interior composition is largely helium or heavier elements, the mean radius deduced for the white dwarfs is 0.01 solar radius, which corresponds to about 0.6 solar mass. The observations of the dependence of line strength on color demonstrate clearly that the ratios of abundance of hydrogen to helium to metals are variable from one type of white dwarf to another. At approximately the same color, white dwarfs can be found with Balmer lines, helium lines, or essentially continuous spectra. Possibly another opacity source exists besides hydrogen; otherwise it seems difficult to understand the hot objects with continuous spectra. A method of observation of broad features has been developed which reliably reveals absorptions of 5 per cent or less in depth, over a wide variety of temperatures.

A method of analyzing the spectra of O-type subdwarfs has been developed and applied by Guido Münch to some of the stars discovered earlier by him. Essentially, it is based on the fact that, over a relatively wide range of effective electron pressure

$P_e$  and temperature  $T$ , for a given H:He abundance ratio, the intensity ratios between lines of He II and H, and between lines of N III and N II, define the same curve in a  $(P_e, T)$  plane. An additional characteristic in this plane independent of the abundances, as is the Inglis-Teller criterion, then defines both  $P_e$  and  $T$ . The analysis of the stars HZ 44 and BD +25° 4655 gives a number abundance ratio H:He = 1:4, while the relative number abundances of the heavy elements represented in the spectra with measurable intensities are N:C:O:Ne:Si = 100:0.5:6:20:6. The effective temperature is found to be 35,000° K, and the surface gravity around  $10^6$ .<sup>8</sup> No direct information is available regarding the absolute magnitudes of these objects, but for assumed masses between 1  $\odot$  and 15  $\odot$ , the corresponding visual absolute magnitudes range from +6.0 to +3.0, respectively. The observational material available on the six stars of this type known at present is now being analyzed.

The subdwarf nature of the blue star BD +25° 2534, found by Dr. A. Slettebak on objective-prism plates taken at Hamburg-Bergedorf, has been verified by G. Münch on 18 A/mm plates taken at Palomar. Although the star had been observed before by Münch and Greenstein, it had not been noticed that, besides the wide and strong Balmer lines, sharp He I lines and a shallow diffuse line of He II at  $\lambda 4686$  also appear. This observation points to the existence of a pseudophotosphere with temperature not as high as would be indicated by the color, where the Balmer lines originate, and of an underlying hot core, where the high-temperature color and spectral characteristics arise. Such a model was suggested in the last annual report by G. Münch to explain the spectrum and color of the blue stars below the horizontal branch of the H-R diagram of globular clusters.

Feige has completed the project for the discovery of faint blue stars on the National Geographic Society-Palomar Observatory Sky Survey plates near the south

galactic pole, and most of the area of the north galactic pole. Thus far a total of 180 faint blue stars between 11 and 16 mag. have been found. Some spectra show that a considerable number of these are the so-called "halo" blue stars. Others, however, are helium-rich subdwarfs, and three definite new white dwarfs are included.

Code has developed a photoelectric scanning spectrograph that has been adapted for both the 100-inch and 200-inch telescopes and has been used for a detailed study of the energy distribution in the spectra of several subdwarfs. All the energy distributions in the infrared of the subdwarfs HD 19455, HD 140283, and HD 201891 correspond to lower temperatures than the temperature inferred from the spectral type or the B-V color. These results are to be understood in terms of weak lines presumably resulting from a low metal abundance. The infrared, where line blanketing is very small, provides a more significant measure of the surface temperature. Relative to normal main-sequence stars, the smaller line blanketing in the subdwarfs leads to a moderate excess in the B-V color and to the larger well known U-B excess. Specifically, HD 19455 has a B-V color corresponding to an F6 dwarf and an assigned spectral type of sdF7, while the spectral distribution in the infrared is that of a G0 dwarf. The subdwarf HD 140283 is similar but somewhat more extreme—a result consistent with the slightly weaker lines as measured in its spectrum.

In order to extend the results obtained to a larger sample of stars a photometer utilizing a red-sensitive photomultiplier was adapted by Code for use on the 100-inch reflector. Several wavelength regions were isolated by means of glass filters and with interference filters. It was found that for stars with the same infrared color index the weak-line objects averaged about 0.1 mag. bluer in the B-V index and 0.25 mag. bluer in the U-B index.

These results have two important ramifications. First, apparently a subdwarf is



not as underluminous as was previously thought; second, any interpretation of a color-magnitude array must be made with due regard for the influence that line blanketing may have upon the color index.

### *Late-Type Stars*

Code has applied his photoelectric scanning spectrograph to the study of the energy distribution of the high-velocity giant Arcturus and of two red giants in M 92. The differences between the observed energy distribution and that of normal giants may be interpreted in the same manner as the subdwarf results reported in the preceding section.

In the spectra of most M-type giants and supergiants, the H and K lines of Ca II show deep absorption reversals ( $H_3$  and  $K_3$ ) superposed on emission components ( $H_2$  and  $K_2$ ). The velocity from  $H_3$  and  $K_3$  always indicates that the absorption gas is expanding from the star. Depending on the height at which the lines are formed, the observed expansion velocity may exceed the velocity of escape, and the matter will be ejected into the interstellar medium. This is true for the M5 II star  $\alpha$  Herculis; it and all other M-type supergiants are losing mass at a rate comparable with their evolutionary time scale. The question arises whether  $H_3$  and  $K_3$  in all M giants arise in envelopes so distended that the observed velocities will suffice for escape. If so, as it was shown last year by Deutsch, the observed number of white dwarfs can be accounted for as the remnants of the massive stars that have condensed into the main sequence during the last 5 billion years.

To investigate the dimensions of the regions responsible for  $H_3$  and  $K_3$  in ordinary M giants, a number of spectroscopic binaries have been observed at 10 Å/mm. If the gas that produces  $H_3$  and  $K_3$  is really circumstellar, and escaping from the system, it cannot follow the orbital motion of the M star; in this event,  $H_3$  and  $K_3$  should be stationary, or nearly so. The periods of M-giant binaries being of the order of a

year or more, and the velocity amplitudes rather small, the spectrograms now at hand do not allow any conclusions. On the possibility that  $H_3$  and  $K_3$  are produced in circumstellar envelopes in G and K giants as well, a few of these stars in binary systems are also being observed on this program.

Coudé spectra of the M2+Ia+ supergiant VV Cephei, made during the total eclipse of its B-type companion, have revealed both circumstellar and interstellar absorption lines at H and K. The interstellar lines have a velocity and strength appropriate to a distance of about 0.8 kiloparsec, in good agreement with Keenan's recent luminosity classification. The circumstellar lines are somewhat weaker than was expected by analogy with other equally luminous M stars. Possibly the B star has an effect on the envelope that persists, in some degree, even during total eclipse.

The system of Mira is similar in some respects. The spectrum of the long-period variable shows a sharp absorption core at K, with a strength about one-third that of  $K_3$  in  $\alpha$  Herculis A. This feature indicates a variable velocity, which correlates with the velocity of the reversing layer but is generally about 2.5 km/sec less. A still weaker absorption core at K has been noted by Joy in the spectrum of Mira B. Its velocity, which appears to be constant, is only slightly less than that of the sharp core in the M star. If both K cores are produced in a common envelope, then the radius of the envelope must exceed 25 astronomical units.

The spectrum of the long-period variable Mira has been found to exhibit a number of remarkable changes from cycle to cycle. As compared with the spectra of ordinary M giants, the absorption features in Mira tend to be the most nearly normal at bright maxima. At most faint maxima, however, the atomic absorption lines are strongly suppressed, especially in the region of the TiO bands. As the star fades, the intensities of many of these lines change in



opposite directions after maxima of these two kinds. The effects are extremely complicated, but often very large. Presumably they are principally stratification effects, but as yet they are not well understood.

An attempt will be made by Deutsch this summer to observe the rapid spectroscopic changes in Mira B found at recent minima of the long-period variable. The companion has been observed visually at each of the last three minima, in roughly the same configuration as at all other reliable observations ( $\rho = 0''.5$ ,  $\theta = 130^\circ$ ). These observations require the abandonment of a period near 14 years, as had been suggested by Parenago. Evidently the motion is very slow, and the mass of the system very low. Another long-period variable with a visual companion is X Ophiuchi. Recent spectrograms of this companion confirm that it is a subgiant which is much less luminous than normal K giants. These two systems suggest that long-period variables are likely to be extremely old stars.

Wilson and M. K. Vainu Bappu have completed the measurements of the line width of the H and K emission lines in 185 late-type stars. As mentioned in the last report, the main result is that a plot of the logarithms of the corrected line widths against visual absolute magnitudes is linear over a 15-mag. range. Another finding is that neither surface temperature nor surface gravity can play much of a role in this functional relationship, which appears to be a relation between a chromospheric phenomenon and the total energy production deep within the star.

A number of important possibilities opened by these observations can be divided into two categories: use of the relationship as a tool for the measurement of absolute magnitude, and information that they provide in regard to the physical processes operating in the atmospheres of these stars.

In considering the first category, the major problem is to refine the calibration, which is a purely empirical matter unless

a theoretical derivation of the width-luminosity relationship can be obtained. So far, additional spectrograms of the four Hyades stars combined with the previous ones yield an average deviation for the method of 0.3 mag. Spectrograms, though not of first quality, of the yellow giants in Praesepe give an average deviation of 0.5 mag. Further observations of both clusters should provide information on measuring error, intrinsic scatter among stars of closely similar luminosities, and the mean line width for these eight stars of known brightness. Even though the calibration of the K-line method is still incomplete, it appears already to be adequate to provide improved information on the yellow and red giants in the solar neighborhood. Under consideration are observations intended to extend knowledge of luminosity functions, color-magnitude diagrams, and masses (through the derivation of improved parallaxes of binaries and physical pairs).

Under the second category, the first problem is to decide on the nature of the emitting layers. Wilson favors the view that they are probably optically thin because of intensity differences between K and H which can be seen in many stars. This evidence is not conclusive, however; the layers may be optically thick. In any event, if the layers are optically thin, then the lines are widened purely by Doppler effect, the measured widths are approximately equal to the Doppler widths,  $\Delta\lambda_D$ , and the turbulence is large. Under these circumstances,  $\Delta\lambda_D \propto L^{1/6}$ , and the only restriction on  $N$  (particles per square centimeter) is that it is never so large that the optical thickness in the line center exceeds approximately 1.

Dr. L. Goldberg, of the University of Michigan, has pointed out that, with optically thick layers,  $\Delta\lambda$ , the measured width, is approximately three times the Doppler width,  $\Delta\lambda_D$ . Thus the real turbulence is only about one-third as large as for thin layers. In this case, however, calculation (unpublished) shows that both

$\Delta\lambda_D$  and  $N$  must vary with luminosity in a prescribed fashion in order to agree with the observations. Thick layers are thus more restrictive on any physical theory seeking to explain the width-luminosity correlation.

Emission at  $H\epsilon$  was first noted by Wilson in the spectrum of Arcturus in 1938. It appears on many spectrograms of the late-type stars used for the foregoing H and K investigation. All these plates were carefully compared with one of Arcturus in order to make a preliminary study of the intensity of the  $H\epsilon$  emission as related to K emission strength, to spectral type, and to absolute magnitude. The chief results are that  $H\epsilon$  intensity increases on the average in the direction  $G \rightarrow K \rightarrow M$ , and that there seems to be a statistical correlation between emission strengths of K and of  $H\epsilon$ . There is not a one-to-one correlation between  $H\epsilon$  and K, since some stars with strong K intensity show no emission at  $H\epsilon$ . Presumably both the Ca II and hydrogen emission are of chromospheric origin, and they may well supply clues to physical properties of the stellar chromospheres.

#### *Variable Stars*

Radial-velocity measurements were completed on spectrograms of the cluster-type variable stars SW Andromedae, DX Delphini, DY Herculis, and DH Pegasi, which had been obtained with the 60-inch telescope during 1955 and 1956 by Tift and Bonsack. Radial-velocity and radius-variation curves were obtained. The velocity curves of DY Her and DH Peg cannot be described as mirror images of the published light-curves for these stars, but the mirror-image relationship does hold for SW And and DX Del.

Tift and H. J. Smith, of Yale, have completed a study of the three-color light-variation and radial-velocity variation for the star T Sextantis, an RR Lyrae star of subclass c.

Wallerstein has completed his study of the velocity curves of Population II

cepheids in globular clusters and in the galactic field. The line emission, and its variations, resembles that in W Virginis. In certain globular-cluster cepheids the absorption lines double, but in others they merely broaden at phases in which W Vir showed line doubling. This material is at present under discussion for the determination of the radius changes, spectral-type variations, and anomalous spectral behavior of the Population II cepheids.

The R Coronae Borealis variable, RY Sagittarii, was found by Greenstein to show variable absorption-line doubling during small light-variations near its maximum, with displaced components up to  $-150$  km/sec. Studies by Greenstein and Merrill of the infrared spectrum of R Andromedae showed that this S star had a circumstellar envelope, visible in certain lines, with about  $-20$  km/sec relative velocity.

Zwicky continued to co-ordinate the search for and the investigation of supernovae conducted at the Lick, Steward, Berne (Switzerland), and Palomar Observatories with the aid of funds from the National Science Foundation and the Swiss Government. During the year two bright supernovae were discovered, one in NGC 2841 by Schürer in Berne, and the other in M 84 by Gates at Palomar. All recent supernovae have been continually photographed by Zwicky with the 48-inch and 200-inch telescopes in four color ranges (ultraviolet, blue, green-yellow, and red) with a view to securing four light-curves corresponding to these ranges. Efforts by Zwicky to investigate theoretically simple models of supernova outbursts have led to results giving promise that both the light-curves and the sequences of the spectra in supernovae of types I and II may become understandable in the near future.

The supernova in M 84 was observed photoelectrically by Baum at the 200-inch telescope. On May 24 its photovisual magnitude was 13.32, and its B-V index was  $+0.80$ . Multicolor observations were also



obtained in order to determine the general character of its spectral-energy distribution.

### *Magnetic Stars*

The results of an 11-year program devoted to the observation of stellar magnetic fields have been summed up in a catalogue of magnetic stars by Horace W. Babcock. Table 1 of this catalogue is a compilation of results for 84 stars for which magnetic fields have been observed by means of the Zeeman effect. Notes are given on the spectra, line widths, abnormal line intensities, and magnetic variations. Table 2 lists 55 stars that probably but not definitely have magnetic fields. Table 3 is a list of 55 stars having sharp lines that give little or no evidence of the Zeeman effect; Table 4 is a collection of 111 stars having lines too broad for magnetic measurements.

The analysis and discussion of the numerous results now available on magnetic stars lead to the following preliminary conclusions. Of the magnetic stars in Table 1 of the catalogue, 1 is a cluster-type variable, 1 is a subdwarf, 2 are of type S, 3 are M-type giants, 7 are "metallic-line" stars, and 64 are sharp-line A-type stars. This variety shows that stellar magnetic fields are in all probability ubiquitous, particularly when it is recalled that only strong coherent fields in sharp-line stars are susceptible of measurement by available methods. Another significant result is that all stellar fields appear to be variable, and the great majority show irregular variations. Stellar magnetic phenomena, like those of the sun, are evidently not simple; rather, the observations reflect complex hydromagnetic fluctuations of the photospheric material. It is legitimate to think of hydromagnetic turbulence in which the energy of the magnetic field is of the same order as the kinetic energy of motion of the gas masses. The statistics of line widths among the rapidly rotating stars of type A are in accord with the supposition that nearly all the sharp-line magnetic

A-type stars are rapid rotators viewed pole-on. As a working hypothesis, it can be maintained that the prevalence of rapid axial rotation plus hydrogen convective zones is responsible, through a dynamo process, for the strong fields of the A-type stars.

Of the magnetic stars found in the course of this survey, many have peculiar and variable line profiles, several are spectrum variables, and six are new spectroscopic binaries. Secular variations in line intensities and in amplitude of magnetic variation have been found in certain magnetic stars. The variety of significant yet unexplained phenomena observed is considerable, and this field deserves increased attention in the future. The interpretation of the stellar magnetic results will undoubtedly be facilitated by the increased understanding of solar magnetic phenomena, and particularly the solar magnetic cycle, that is accruing from current studies.

The star HD 125248 reverses its magnetic field with a period of 9.3 days, and shows synchronous changes in line strength, line width, and radial velocity. By a kind of harmonic analysis, Deutsch has shown that it is possible to derive a rigidly rotating configuration which satisfies the observations in any one cycle reasonably well. Superposed on the 9.3-day cycle, however, is another, much slower, variation in radial velocity, which has heretofore prevented the compilation of observed velocities to form mean curves.

From coudé observations accumulated over a 10-year interval, by Deutsch and H. W. Babcock, it has now been found that the secondary velocity variation has a period of about 1670 days. The amplitude of this long cycle is about 7 km/sec, or more than twice that of the 9.3-day cycle. The long cycle is probably due to Kepler motion in an eccentric orbit about an invisible companion.

When the orbital velocity variation is removed, the observations may be combined to yield mean velocity curves which represent the motions due to rigid rotation.



One such curve is required for the lines of Eu II, Gd II, and Ce II; a second for the lines of Cr I and II; and a third for the lines of Fe I, Fe II, and Ti II. Since these mean curves make use of many more observations, they are much better determined than the velocity curves previously used for the harmonic analysis. Accordingly, the harmonic analysis will be repeated by Deutsch in an attempt to improve the fidelity of the map that has been derived.

Spectrograms were obtained by Bonsack with the 60-inch telescope of the spectrum variable 56 Arietis. They were measured to determine the radial velocities of the individual lines as a test of the rigid magnetic rotator model for this star. Analysis of the wavelength and intensity variations of the lines showed that the star can be represented as a rigid rotator of period 0.73 day.

Because of the unusually strong Si II lines, 21 Aquilae is placed in the group of "silicon-helium" peculiar A stars although it is classified as B8 from the He I lines in its spectrum. Its spectrum lines are unusually narrow, so that wavelengths can be measured with good accuracy. Combining Miss Burd's measurements of plates taken by H. W. Babcock with data from a plate taken at the McDonald Observatory, G. R. and E. M. Burbidge have obtained evidence for a systematic shift between the singlet and triplet lines of He I. Although some part of this may be due to Stark effect, comparison with the laboratory measures of isotope shifts between lines of He<sup>3</sup> and He<sup>4</sup> suggests that there is a reasonable probability that the major part of the shift may be due to the presence of He<sup>3</sup> in the star's atmosphere in an amount comparable with that of He<sup>4</sup>.

The presence of He<sup>3</sup> may be explained by a theoretical treatment, carried out by G. R. Burbidge and by W. A. Fowler and E. M. Burbidge, of the Kellogg Radiation Laboratory, of nuclear reactions on the surfaces of magnetic stars. It appears that a flux of neutrons will be produced by

reactions between accelerated protons and light nuclei, and will be captured by cool hydrogen to form deuterium. Reactions between deuterons and protons will either build He<sup>3</sup>, or free the neutrons again, so that eventually they may be captured by elements in the iron group to build heavy nuclei.

### *Globular and Galactic Clusters and Stellar Evolution*

Schmidt has started an investigation of the color-magnitude diagram for the galactic cluster NGC 6939. The cluster appears to be relatively old and has an incipient Hertzsprung gap. More photoelectric standards are being obtained.

Photographic material has been obtained for the galactic clusters NGC 2269, 2309, 2311, 2367, 2401, 2453, and 6834. Poor observing conditions in the winter prevented the establishment of photoelectric standards in these clusters.

Schmidt carried out photographic, photoelectric, and spectroscopic observations on a small condensation of stars at R.A. 19<sup>h</sup> 14<sup>m</sup> 35<sup>s</sup>, Dec. +15° 13'.7. The diameter is 1'×2', and the tenth-brightest star is  $m_{pg}=16.5$ .

Study of color-magnitude diagrams for a number of star clusters was continued by Sandage during the report year in connection with the problem of stellar evolution. Because of the importance of the ultraviolet excess shown by stars with  $B-V \geq 0.5$  in the globular clusters M3 and NGC 4147 (reported last year), Sandage and Walker did three-color photometry on the UBV system for 200 of the brighter stars in M 92. Photoelectric observations were made on 8 nights with the 60-inch telescope, and a special series of photographic plates were taken with the 100-inch. The measurements, which have been completed, indicate that the giant stars in M 92 also show the  $\Delta(U-B)$  excess of about 0.3 mag. in agreement with the value found for stars in M 3 and NGC 4147. Stars on the horizontal branch, however,

show an ultraviolet deficiency of nearly  $\Delta(U-B)=0.15$ , again like stars in M 3, M 13, and NGC 4147. It is therefore becoming clear that the energy-distribution curves for globular-cluster stars differ in a fundamental way from curves for stars in the solar neighborhood, and the difference, which shows up in the three-color photometry, provides an easy means to search for globular-cluster-like stars in the general field. The differences in the U, B, V values between globular-cluster stars and normal field stars are believed to be due to differences in chemical composition and the resulting blanketing of the continuum by the absorption lines in the blue and ultraviolet region of the spectrum. A special study of the blanketing effect was begun by E. M. Burbidge, G. R. Burbidge, and Sandage with the globular-cluster problem in mind. The study is reported in more detail in another section of this report.

The globular cluster NGC 5897 was placed on the three-color program because a spectrogram by Deutsch showed that this cluster had very weak absorption lines and therefore is expected to have a large ultraviolet excess. Photoelectric observations with the 100-inch and photographic plates with the 200-inch were obtained for this cluster by Sandage. Schmidt is reducing the photographic material, but the results are not yet available. Other clusters on the current program include NGC 6356, which is one of the W. W. Morgan strong-line globular clusters near the galactic nucleus; NGC 6712, a globular cluster situated in the Scutum star cloud; NGC 7789, an old galactic cluster probably of the M 67 type; NGC 7788, which has the three classical cepheids CF Cassiopeiae, CE a Cas, CE b Cas associated with it; and NGC 7790, which forms a double cluster with 7788. Sandage has obtained photoelectric calibrations and photographic plates for all these clusters with either the 60-inch or the 100-inch telescopes. The measurements are almost complete for most of the clusters.

The work on NGC 7789 is a joint project with E. M. Burbidge.

The cluster NGC 1866 in the Large Magellanic Cloud is under study by Arp and Sandage in collaboration with Dr. A. D. Thackeray, of the Radcliffe Observatory, Pretoria, South Africa. This cluster is globular in appearance but has a color-magnitude diagram that seems at this stage of the investigation to resemble that of M 11. Its importance lies in the fact that 9 cepheids with periods ranging from 2.64 to 3.52 days are associated with the cluster. All these cepheids have the same apparent magnitude and therefore are at the same place in their evolutionary history. The details of the connection of the cepheids with the nonvariable stars in NGC 1866 promise to clarify our ideas of how cepheid variables fit into the evolutionary picture. Photoelectric calibration to  $V=19.0$  was completed by Arp while he was in South Africa; Thackeray has obtained an extensive series of plates with the 74-inch Radcliffe reflector; Sandage has started measurement of the plate material. At the present writing, NGC 1866 appears to be one of the most important clusters in the sky for the evolutionary problem.

Several years ago, E. E. Salpeter, of Cornell, derived a theoretical luminosity function giving the distribution of stars along the main sequence at the time of their formation. This creation function is of interest because with it can be predicted the number of stars at any given luminosity which have been formed in the lifetime of the Galaxy. Because of its significance, observational checks on the Salpeter function are necessary. Sandage showed that the luminosity functions in each of the open clusters  $\eta$  Persei, Pleiades, Coma Berenices, Hyades, and Praesepe agree well with the Salpeter creation function, and this agreement supports the argument that the peculiar form of the van Rhijn luminosity function for stars in the general field brighter than  $M_v = +3.5$  is due to stellar evolution.

Sandage derived a new luminosity func-



tion for M 3 which predicts a total mass for M 3 of  $2.45 \times 10^5 M_{\odot}$ , a total number of stars of  $5.9 \times 10^5$ , and a total number of white dwarfs of  $4.8 \times 10^4$ . By means of the Salpeter creation function it was estimated that the stars which are now white dwarfs in M 3 have shed a mass equal to  $1.1 \times 10^5 M_{\odot}$  in the form of gas as they evolved from the main sequence to the white-dwarf stage. This mass has escaped from the cluster into the interstellar medium. Similar considerations for E galaxies show that the gas formed by the shedding process has probably not escaped and makes up about 1/200 of the total mass of the E galaxy. Osterbrock has suggested that this mass may be the origin of the  $\lambda 3727$  [O II] emission from the E systems.

Semiempirical evolutionary tracks were computed by Sandage for the stars in M 3 and M 67. M 3 and M 67 are clusters of about the same age ( $\sim 5 \times 10^9$  years), but the stars in each cluster follow very different tracks of evolution in the giant region even though they are of about the same mass. Presumably the track differences are due to differences in the chemical composition of the two clusters. The method of obtaining the tracks of evolution for individual stars utilizes the information contained in the observed luminosity functions and color-magnitude diagrams of clusters. The evolutionary tracks, the time scale for evolution along these tracks, and the fraction of the total mass exhausted of hydrogen at each evolutionary stage are determined. These semiempirical results were compared with the predictions of the Hoyle-Schwarzschild (HS) theoretical evolving models, and Sandage showed that the time scale of the HS models gives nearly the correct luminosity function for M 3 except at the top of the giant branch, where the HS time scale is too fast by a factor of 3. The lifetime of the RR Lyrae stars is estimated to be  $8 \times 10^7$  years. This figure gives an expected rate of change of period for these variables due to evolution of  $\Delta t/t = 2.40 \times 10^{-11}$ , which is 0.1 second of time per century. The final result of the

study shows that stars in M 3 completely exhaust their energy store of  $1.60 \times 10^{52}$  ergs in their lifetime as they evolve from the main sequence, through the giant stage, and finally to the white dwarfs. But stars in M 67 exhaust only 37 per cent of their energy store. Since there can be no internal energy reservoir in the white dwarfs, this result suggests that mass loss must occur from stars like those in M 67 before they become white dwarfs. This conclusion is supported empirically by Deutsch's study of the giant  $\alpha$  Herculis reported last year.

### *Chemical Composition of Stellar Atmospheres*

Under the sponsorship of the Physics Division of the U. S. Air Force, Office of Scientific Research, a project entitled "Stellar Composition and Related Nuclear Processes" has been established at the Mount Wilson and Palomar Observatories under the direction of Greenstein. The goal is to increase the astronomical data relevant to theories of the origin of the elements, as well as to bring together a group of nuclear physicists and theoretical astrophysicists interested in aspects of this fundamental question. It is generally accepted now that certain stars show evidence for the current formation of elements, for example most obviously the element technetium in S stars, originally discovered by Merrill. In addition, the thermonuclear conversion of hydrogen to helium, and the burning of helium to carbon, in stars has been definitely established. These processes occur in certain parts of the evolution of stars, and should be correlated with studies of stellar-interior theory. The group under Air Force sponsorship will apply more advanced theoretical techniques to the determination of the abundances in normal and peculiar stars, with the hope of correlating these results with nuclear data and theory. The general question of the possible evolutionary trend in the abundance of heavy elements with time in our own Galaxy will also be studied.



Abundance analyses for such elements in carbon-rich stars, S stars, stars with abundant rare earths, very old and very young main-sequence and giant stars, and young and old subgiants will be among the goals of this research. It is hoped that the study will be continued for several years.

The two-lined spectroscopic binary +74°493, a high-velocity dwarf, has been studied by Greenstein in collaboration with Drs. Margherita Hack and Otto Struve, of the University of California. The masses seem to be close to those of normal main-sequence G dwarfs; in other words, high velocity does not necessarily mean in the dwarfs a deviation from the mass-luminosity relation. A spectrophotometric analysis showed that the lines may be slightly weaker than normal for similar Population I dwarfs, indicating a slight underabundance of the metals.

A detailed study of line intensities of the metals, CN, and CH has been made by Greenstein and Dr. Philip C. Keenan, of the Perkins Observatory. Fourteen giants near spectral type G8 of both high and low velocities have been studied. The deduced reduction in the number of metallic atoms in high-velocity stars is by a factor of about 0.4 to 0.6; the effective number of CN molecules is reduced to about 0.06 normal, and CH is hardly affected. Two new apparently carbon-poor stars have been found. One of the high-velocity stars,  $\upsilon^2$  Cancri, is shown to be of normal composition; it is therefore a runaway evolved Population I star.

Further spectra of the brightest stars in the globular clusters M 13 and M 92 have been obtained by Greenstein at 18 A/mm with exposures running from two to three nights. The M 13 spectra resemble those of high-velocity stars in the solar neighborhood; the M 92 spectra show very weak but numerous metallic lines corresponding to a quite low excitation temperature. A quantitative analysis of these stars is planned.

A survey of early R stars at dispersion 4 and 7 A/mm is being carried out by

Greenstein for the study of the atomic-line differences between the hydrogen-rich and hydrogen-poor carbon stars.

Work was begun by Bonsack on an extensive program to survey a variety of stars of type K to determine the possible variations in the abundance of lithium, and a number of stars of type A to study beryllium.

The comparison of the spectrum of the Ba II star HD 46407 with that of the standard G8 III star  $\kappa$  Geminorum has been continued by G. R. and E. M. Burbidge. The theoretical curves of growth used were those by Wrubel for a Milne-Eddington atmosphere. Conditions in the standard star  $\kappa$  Gem and in HD 46407 were found to be very similar, as had been anticipated. There were no detectable differences between the two stars in  $T_{\text{exc}}$ , in the degree of ionization, or in the total velocity (thermal and turbulent). From a preliminary comparison with the sun,  $T_{\text{exc}}$  was found to be 3900° C; with  $\log P_e = -0.3$ ,  $T_{\text{ion}}$  was found to be 4200° C; the velocity parameter was 4 km/sec.

Relative abundances of the following elements were determined: sodium, magnesium, aluminum, silicon, calcium, scandium, titanium, vanadium, chromium, manganese, iron, cobalt, nickel, strontium, yttrium, zirconium, barium, lanthanum, cerium, praseodymium, neodymium, and samarium; also less certain values were found for copper, zinc, germanium, niobium, molybdenum, ruthenium, europium, gadolinium, ytterbium, and tungsten. Carbon has a slightly greater than normal abundance in HD 46407. The elements from sodium through germanium were found to have the same abundances in the Ba II stars and in  $\kappa$  Gem, apart from barely significant slightly increased abundances of aluminum and scandium in HD 46407. Most of the heavier elements, from strontium onwards, however, were found to have abundances of the order of 10 times those in the standard star.

The results have been discussed in the context of a theory of the stellar origin of

the elements in the universe; they are found to give good support for it. On this theory certain isotopes of the elements heavier than iron are built by a slow neutron-capture process in the interiors of red giant stars. Abundance peaks are produced at nuclei having a magic number of neutrons, e.g. strontium, yttrium, zirconium, barium, lanthanum, cerium, praseodymium, and neodymium, all of which are overabundant in HD 46407. Europium, so prominent in peculiar A stars with magnetic fields, is not built predominantly by neutron capture on the time scale supposed to occur in red giant stars, and its abundance in HD 46407 is no larger than in  $\kappa$  Gem.

Coudé spectrograms have been obtained by G. R. Burbidge with the 100-inch telescope for a continuation of the program for the determination of heavy-element abundances in cool stars. The identification by Merrill of Tc I in a carbon star indicates that element synthesis through a slow neutron-capture chain occurs in these stars as well as in S and Ba II stars. A number of carbon stars have been observed, including "normal" stars with  $C^{13}$  bands, hydrogen-poor stars with no  $C^{13}$ , and CH stars.

Sandage and G. R. and E. M. Burbidge have started a program for the measurement of the blanketing effect in a number of standard stars in the range F7 to G2, and in the Population II star HD 19445. The latter has a low abundance of most elements, relative to hydrogen, and consequently weak spectral lines. In a two-color plot of  $U-B$  against  $B-V$ , this star has an ultraviolet excess. It lies close to the line on which H. L. Johnson and Sandage have shown the stars in M 3 to lie. The program is to see whether the difference in blanketing between HD 19445 and a normal star of the same effective temperature would move the star in the  $U-B$ ,  $B-V$  plane so as to account for the ultraviolet excess. It will certainly account for part of it, but whether there is a remainder needing some other explanation must be

determined. If blanketing can account for the whole effect, then we shall be able, in principle, to use two-color measures to deduce whether the elements calcium, iron, etc., in a distant group of stars are underabundant, relative to hydrogen, and by how much. All the observations, consisting of 10 Å/mm spectrograms covering the range  $\lambda 3300$  to  $\lambda 6300$ , have been obtained with the 100-inch telescope. Almost all the tracings have now been completed, with the Babcock microphotometer, and their measurement with a planimeter is now under way.

### *Nuclear Reactions in Stars*

G. R. Burbidge and F. Hoyle, and Drs. W. A. Fowler and E. M. Burbidge, of the Kellogg Radiation Laboratory of the California Institute, have continued their work on the synthesis of the chemical elements in the stars. They find that eight processes, as follows, are necessary to account for the abundances of all the 327 isotopes found in the solar system: (1) Hydrogen burning is responsible for the majority of the energy production in stars. This process synthesizes helium, and, when it occurs in a mixture of hydrogen with other elements, it builds all those isotopes of carbon, nitrogen, oxygen, fluorine, neon, and sodium that are not built by process 2. It occurs in main-sequence stars and in shells around the cores of giants. Its results may be seen in hydrogen-exhausted hot stars, some carbon stars, WN and other nitrogen-rich hot stars, and some white dwarfs. (2) Helium burning builds  $C^{12}$  from helium and, by further  $\alpha$ -particle addition,  $O^{16}$ ,  $Ne^{20}$ , and perhaps  $Mg^{24}$ . Its onset occurs in the cores of red giant stars, and it presumably continues in stars in later evolutionary stages. Its results may be visible in some carbon stars and in WC stars. (3) The  $\alpha$  process builds, through charged-particle interactions, the rest of the four-structure nuclei  $Mg^{24}$ ,  $Si^{28}$ ,  $S^{32}$ ,  $A^{36}$ ,  $Ca^{40}$ , and probably  $Ca^{44}$  and  $Ti^{48}$ . (4) The  $e$  process builds the elements comprising the iron peak in the abundance curve (vana-



dium through nickel). It occurs at very high temperatures and densities, when conditions of statistical equilibrium are set up. Both it and the  $\alpha$  process are thought to occur shortly before a star explodes as a supernova. (5) The  $s$  process is a slow neutron-capture chain which builds many of the isotopes in the ranges  $23 < A < 46$  and  $63 < A < 209$ . It is thought to take place in the interiors of red giant stars, when neutrons are produced, and to be responsible for the observed anomalies in S stars, Ba II stars, etc. (6) The  $r$  process is a rapid neutron-capture chain, occurring on a very short time scale, and is thought to take place in supernovae. It will produce a large number of isotopes in the range  $70 < A < 209$ , and uranium and thorium. It may also build a few lighter isotopes not built by other processes, e.g.  $S^{36}$ ,  $Ca^{46}$ ,  $Ca^{48}$ . The decay of radioactive  $Cf^{254}$ , built by this process, is thought to be responsible for the exponential light-curve of some Type I supernovae. (7) The  $p$

process is a proton-capture or photoneutron process, also thought to occur in some supernovae. It builds the remaining isotopes in the range  $63 < A < 209$  which are proton-rich, have abundances 0.01 to 0.001 times the near-by normal and neutron-rich isotopes, and cannot be built by either the  $s$  or the  $r$  process. (8) The  $x$  process, not properly worked out yet, must be responsible for building deuterium, lithium, beryllium, and boron, which are unstable in hydrogen burning in stellar interiors. There may be some production in stellar atmospheres in magnetic stars (including all stars with flare activity), and deuterium may be made in some supernovae when a large flux of neutrons impinges on an expanding, relatively cool envelope in which hydrogen has not been exhausted.

Further work on the  $x$  process is going on at present. Observations have been made with Dr. Philip C. Keenan, of the Perkins Observatory, and theoretical work is under way with W. A. Fowler.

## GASEOUS NEBULAE

### *Internal Motions and Radial Velocities*

In past years, Guido Münch and Wilson have reported on an extensive series of observations of the internal motions in the Orion nebula using a multislit on the 72-inch camera of the 200-inch coude spectrograph. During the current year they have extended these observations to fainter regions of the nebula by using the multislit with the 36-inch camera.

All plates have now been measured and reduced by Miss Flather and Mrs. Coffeen for radial velocities of the [O II], H, and [O III] lines. A large number of line profiles have also been determined, and the material is being analyzed. The aspect of the problem related to the verification in the nebula of the predictions of the equilibrium theory of turbulence at high Reynolds numbers has been studied. In good agreement with results derived by Dr. S. von Hörner from radial velocities determined at the Lick Observatory, Münch and Wil-

son find that the mean square difference between two points in the nebula separated by a distance  $d$  varies nearly as  $d^{2/3}$ , for values of  $d$  between 1" and 60". At variance with von Hörner's conclusions, however, they find that this statistical relation cannot be taken as a proof that the Kolmogoroff law is satisfied in the nebula, for, if it were, the line widths predicted would be 3 times smaller than those observed. If Kolmogoroff's law were satisfied, the averaging effect along the line of sight involved in the observations should be negligible, and the nebula would be required to have the shape of a thin sheet of matter in the plane of the sky. They find such a configuration inadmissible in the light of direct observational evidence, such as the appearance of the line  $2^3S-3^3P$  of He I in absorption in the spectra of the illuminating stars and also the reddening of the Trapezium cluster. The failure of the Kolmogoroff law to describe the state of



motion of the nebula is due to the compressibility effects. The spectra show many areas in the nebula where the lines appear distinctly as double, suggesting the existence of discontinuities in the flow, produced by shock waves. Münch and Wilson visualize these shock waves as a result of the interaction of the expanding H II region with the surrounding cold material.

An investigation of the radial velocities in the Cygnus loop has been completed by Minkowski. The average picture found is that of an expanding incomplete thick shell. The velocity of expansion is 65 km/sec at the inner boundary with a diameter of about 80' and 115 km/sec at the outer boundary with a diameter of about 170'. This picture resembles in many ways the appearance of IC 443, which is obviously an object of the same type as the Cygnus loop. If the velocity of expansion at the outer border is combined with the outward motion of 0''.03 per year found by Hubble, a distance of 770 parsecs is obtained. The diameter of the main part of the nebula is then 40 parsecs; faint matter in the south extends to a distance of 35 parsecs from the center.

The absence of an exciting star suggests that the excitation of the emission spectrum in the Cygnus loop is collisional. The observational decision on the type of excitation depends essentially on the ratio of the intensities of  $H\alpha$  relative to  $H\beta$  for which conflicting results had been obtained by various observers. New measures by Minkowski confirm the result by Pikelner that the average ratio  $(H\alpha + [N II]):H\beta$  has the value 6.1. The  $[N II]$  lines, however, have considerable strength, and the average value of the ratio  $H\alpha:H\beta$  is 3.4, with local variations up to 5.4. These values are consistent with the interpretation that the excitation is collisional. The lower values of the ratio conform to electron temperatures of the order of 100,000°, which is in general agreement with the temperatures found from the relative intensities of the  $[O III]$  lines  $\lambda 4363$  and  $\lambda 5007/4959$ .

The fact that the excitation is collisional

supports Oort's suggestion that the Cygnus loop is a shell originally ejected at a high velocity and decelerated by collisions with interstellar clouds. In order to form an object like the Cygnus loop in a region of average interstellar density ( $N_H = 0.01$  to 1), the shell must have a momentum of the order of  $10^{43}$  cm. g. sec<sup>-1</sup>. This value is too high for shells ejected by ordinary novae and even by supernovae of Type I, such as the Crab Nebula, but it appears possible that such a value pertains to the shells of supernovae of Type II. The Cygnus loop and other objects of this type thus may be the hitherto unobserved remnants of these supernovae.

A preliminary discussion of the observations of the radial velocities of the bright filaments in the Crab Nebula has been prepared by G. Münch. It was found that many of those filaments that by their radial velocities can be considered as a unit are oriented at right angles to the direction of the magnetic field, as determined by Hiltner from polarization observations of the synchrotron radiation. This observation suggests that the filaments move in a "force-free" field, in which the electric currents are parallel to the magnetic field. The radial velocities of the brighter central filaments also show that their motion is not one of uniform expansion from the star supposed to be the remnant of the supernova. Study of the large-scale geometry of the filamentary mass disclosed that the direction of the major axis of the nearly elliptical outer boundary is that of the galactic equator, to the degree of precision with which such major axis can be determined. The importance of the role that the prevalent galactic magnetic field plays in determining the present structure of the shell thus becomes apparent. The outer galactic magnetic field being responsible for the ellipticity of the shell, it follows that the expansion velocity of the shell at present may have values along the lines of force differing from values at right angles to them. The distance of the Crab Nebula, determined on the assumption of

the equality of the cross motions with the radial velocities, may have to be revised and increased upward. Further observations of the fainter filaments are being planned to elucidate this point.

Because of the renewed interest in the intensity changes in the continuum of the Crab Nebula, the continuum was regularly photographed by Baade in the range  $\lambda 5300$  to  $\lambda 6400$ . No moving wisps were observed during the fall of 1956.

In continuation of observations going back to the late 1930's, the shells of Nova T Aurigae (1891), Nova Cygni (1920), and Nova Herculis (1936) were photographed by Baade at the prime focus of the 200-inch. The progressive expansion of all three shells is very conspicuous in the blink comparator.

Radial velocities of 101 faint planetary nebulae have been determined by Minkowski; almost all of them are for objects which are within  $10^\circ$  from the position of the galactic center. In this region the velocities range between about  $-300$  and  $+300$  km/sec, showing the presence of a large velocity dispersion in the central part of the Galaxy. The observations are still being continued, but the available results are already adequate for a general discussion of the motions of planetaries, which Schmidt has started.

### *Densities of Nebulae*

Osterbrock continued measurements of the intensity ratio of the two components of the [O II]  $\lambda 3727$  doublet in order to determine the electron densities in gaseous nebulae. Measurements of this ratio show that the density in the brightest filaments of the Crab Nebula is of the order of 1000 electrons/cm<sup>3</sup>. The total volume of such filaments, estimated from Baade's direct plates of the nebula, is approximately  $8 \times 10^{51}$  cm<sup>3</sup>, and the resulting mass of the system of brightest filaments is about 0.02 solar mass. Rough estimates of the densities and volumes of the fainter filaments can also be made, leading to a total mass of the whole filamentary system of the

Crab Nebula in the range 0.05 to 0.1 solar mass.

Plates were also obtained for measurement of the  $\lambda 3727$  intensity ratio in a number of planetary nebulae, particularly IC 418, NGC 6720, and NGC 7293. These observations were planned to study the spatial-density variation in the various planetaries, for the electron density gives a good measure of the mass density in these objects. Reductions of the NGC 6720 observations show that in this well known ring planetary all the regions that emit  $\lambda 3727$  have essentially the same density, about 1000 electrons (or hydrogen ions) per cm<sup>3</sup>. Additional observations of the  $\lambda 3727$  ratio in the Orion nebula were obtained, to complete the study of the large-scale structure of this object.

Several diffuse nebulae in which a dark cloud is ionized by an O star outside the cloud were studied by Osterbrock. The ionized material, seen in emission, lies mostly between the star and the dark cloud, and is sharply bounded on the side toward the cloud by a bright rim, while on the other side it fades out gradually. In each of these nebulae, long-exposure schmidt photographs taken in H $\alpha$  show that there is parallel filamentary structure, approximately perpendicular to the bright rim, in the emission nebulosity (these striations were observed some years ago in IC 434, the brightest member of this group, by Duncan, with the 100-inch telescope). These parallel bright filaments must result from regions of high density in the original cloud, which are drawn out by expansion without being mixed appreciably. There are two possible interpretations for the fact that the filaments are long but remain unmixed: either there is a magnetic field that prevents motions perpendicular to its own direction in all these objects, or else the mechanism that generates turbulence in most diffuse nebulae cannot operate in these objects, perhaps because they are expanding into regions of very low density.



## GALAXIES

*The Andromeda Galaxy (Messier 31) and Other Members of the Local Group*

During the past year the photovisual observations of the cepheids in the outer field of the Andromeda galaxy, 96' south preceding the nucleus, were concluded by Baade. Combined with the earlier photographic series of plates they should furnish the necessary data about color excesses and absorption in this field. Still under way is a survey of this same field for faint red variables which were below the plate limit of the 30-minute photographic series at the 200-inch but show up in remarkably large numbers on photovisual and red plates of long exposures. These faint red variables are clearly members of the Population II which pervades the whole disk of the Andromeda galaxy (the "interarm" population). They deserve further investigation because they should throw much needed light on the characteristics of the interarm population of our own Galaxy.

Most of Baade's observing time during the past year was devoted to the dwarf E galaxies of the local group which as typical representatives of the pure Population II are of special interest. Four of them (the Sculptor, Ursa Minor, Draco, and Leo II systems) are close enough to reach the cluster-type variables with the large modern telescopes. Although all four are E galaxies of very low luminosity, the cluster-type variables appear in them in large numbers (on the average more than 200 cluster-type variables per system). Type II cepheids with periods longer than a day are rare in these dwarf systems. The Draco system, for instance, contains only two, and the same number was found by the Pretoria observers in the Sculptor system. But with increasing stellar content their number increases rapidly; examples are the Fornax system and the Leo I system north of Regulus.

Miss Swope has finished the investigation of the variables in the Draco system. She also completed the photographic and

photovisual measurements for the color-magnitude diagram of this system. The results can be presented in final form as soon as the photoelectric sequence in Messier 13, which has been measured by Dr. H. L. Johnson, of the Lowell Observatory, and which has been transferred to the Draco system, becomes available. Since enough plates have been obtained for the investigation of the variables in the Leo II system, only the Ursa Minor and Leo I systems remain under observation.

The study of the stellar contents of M 33, reported last year, has continued. Humason and Sandage began a search for the red supergiants which are expected from evolutionary considerations to accompany the blue O and B type stars in the spiral arms. Over 1000 red supergiants were found in M 33 by Humason by blinking pairs of blue (103a-O+GG 13) and yellow (103a-D+GG 11) plates taken with the 200-inch. A special field south preceding the nucleus was chosen to study these stars, and plates taken sporadically during the season indicated that many of these red stars are irregular variables. They may be the extragalactic analogues of the M supergiants found in  $\eta$  and  $\chi$  Persei, for example. Photometric measurements on the UBV system are in progress with a standard photoelectric sequence set up by Dr. H. L. Johnson in M 33 several years ago.

Plates in blue and yellow wavelengths for a number of other galaxies (NGC 6822, Sextans Dwarf, Leo Dwarf, W-L-M Dwarf, NGC 2403, M 101) have been obtained for a similar study of red supergiants. Blink surveys show that the red supergiants are indeed present in these galaxies as well as in M 33.

Schmidt has computed a model of the distribution of mass in M 31. It is based on recent results obtained by Dutch observers from observations at 21-cm wavelength, and gives a total mass of  $3.4 \times 10^{11}$  solar masses. About 50 per cent of the mass has a mass-luminosity ratio of around 27;



the ratio for the whole system has about the same value.

### *Studies of Individual Galaxies*

Osterbrock began obtaining spectra at a dispersion of 66 Å/mm of those elliptical galaxies already known from the observations of Humason to have [O II]  $\lambda 3727$  in emission. Only a few galaxies have been observed to date, but several interesting preliminary results have appeared. In all the galaxies studied, except a single E0, the material emitting  $\lambda 3727$  is in rapid rotation. One E0 and one E1, both very nearly round objects, as well as several more-elongated objects, show this rotation of the interstellar matter quite definitely. That the  $\lambda 3727$  lines are resolved into two components in only one object indicates quite high turbulent velocities as well as systematic rotational velocities for the interstellar matter near the nuclei of these elliptical galaxies. Among the ellipticals known to have  $\lambda 3727$  in emission, there are wide variations in the strength of the line, but it is invariably so strong at the 66 Å/mm dispersion that possibly all ellipticals will have detectable  $\lambda 3727$  at this dispersion.

The blue galaxies recently discovered at Tonantzintla by Dr. G. Haro are being observed both spectroscopically and directly by Guido Münch. Among those already observed, an appreciable fraction have been found to have regular geometry, resembling that of early and intermediate spirals. When spiral arms can be seen, as in NGC 263 and NGC 2415, they appear thicker and with brighter condensations than in typical spirals. Their spectra show the emission lines characteristic of planetary nebulae, superposed on a continuum corresponding to an early spectral type. Strong Balmer lines in absorption are prominent, and in one case the line He  $\lambda 4471$  has been measured in absorption. The emission lines and early-type spectrum extend through the entire body of the systems, with increased relative strengths of the emission lines in the nuclei and in the con-

densations. The components of the [O II] doublet are clearly resolved, indicating that, unlike the condition in the normal ellipticals, the random motions of the gas producing them are small. The relative intensities of the [O II] lines indicate number densities of free electrons of the order of  $10^3 \text{ cm}^{-3}$ .

Code has made scans of several elliptical galaxies in the Virgo cluster with his photoelectric scanning spectrograph. His curves should provide further information on the energy distribution in the spectrum of elliptical galaxies and on their possible stellar content.

Tifft has begun the photoelectric photometry of the brighter galaxies in four colors in the range  $\lambda 3400$  to  $\lambda 6000$  Å. This work will be extended to the red and infrared this coming year. Field galaxies of nearly all types, with special emphasis on those for which W. W. Morgan has derived spectral types, are being obtained. The Coma and Virgo clusters are included for comparison. The hope is that the photometric system will be calibrated in absolute energy units. The goal is a study of the variations of color between galaxies and within galaxies, as well as an attempt to synthesize the observed colors in terms of the stellar populations present.

Zwicky has continued the investigation of pairs and groups of galaxies that are interconnected by luminous intergalactic filaments. All types of galaxies have been found to be thus linked by luminous matter, usually blue, suggesting clouds of sub-luminous blue stars whose absolute photographic magnitude is greater than  $M_p = 0$ . Most of the double nebulae photographed originally by Pease with the 60-inch reflector were rephotographed and found to show faint countertides on one or on both components. The absence of countertides would lead the investigator to suspect strongly that he was dealing with optical doubles whose differences in radial velocities should not be used for a determination of the masses involved.

### *Catalogues and Statistics of Galaxies*

Work on the catalogue of galaxies brighter than the apparent photographic magnitude  $+15.5$  has been continued by Herzog and Zwicky with a grant from the Office of Naval Research. Charts are being prepared for publication of all results in the strip from  $\alpha=7^h$  to  $17^h$  between  $\delta=-10^\circ$  and  $+20^\circ$  with all the measured galaxies indicated by various symbols giving the photographic magnitudes in ranges of 1 mag. Each chart is on the scale of the 48-inch photograph, and for the convenience of the users is centered near the center of a 48-inch Sky Survey chart, 1950 co-ordinates being used. The catalogue, when completed, is expected to give the magnitudes and positions as well as certain other data for 35,000 galaxies.

In preparation for a study of the statistics of galaxies and clusters of galaxies the strip between  $\alpha=12^h$  and  $14^h 40^m$  from  $\delta=+5^\circ$  to  $+15^\circ$  was completely covered by Zwicky with 27 fields at the 48-inch, each field having been photographed on 103a-O with exposures of 3.5 and 10 minutes and on 103a-D behind yellow Plexiglass filter with exposures of 5 and 15 minutes. Extensive counts covering large numbers of galaxies in a large cap around the north galactic pole have confirmed the previous result that fields containing many near-by galaxies (large near-by clusters) contain relatively few distant galaxies. If  $n_b$  is the number of galaxies brighter than  $+15.5$  on one of the charts of 36 square degrees of our new catalogue, and  $n_f$  is the number of galaxies in the approximate range from  $+15.5$  to  $+19.0$  counted on good red 48-inch schmidt plates, we have approximately  $n_b \times n_f = B$ . The value of  $B$  decreases rapidly when we come near the belt of interstellar obscuration, but  $B$  is approximately a constant  $= 5 \times 10^6$  in a large cap of  $40^\circ$  radius around the north galactic pole. The lowest values of  $n_f$  are obtained near the centers of the near-by clusters in Virgo and Coma, suggesting that intergalactic dust producing obscuration to the

amount of 0.25 to 0.5 mag. is locally concentrated in the central regions of these clusters.

The nonuniformities in the distribution of galaxies are exceedingly pronounced, both locally and integrally. Zwicky believes that this can be understood only on the basis of the assumption that clustering of galaxies is a universal phenomenon and that the apparent distribution of galaxies is affected in an intricate way by the effects of both interstellar and intergalactic obscuration. These circumstances make it difficult to arrive at any definite conclusions regarding the real distribution of galaxies throughout cosmic space.

### *Clusters of Galaxies*

About two dozen rich globular clusters of galaxies were chosen by Zwicky for further detailed investigation concerning total population, radial distribution, structural index and distribution index, distance, symbolic velocity of recession, and internal velocity dispersion.

The faintest among these clusters, barely recognizable on red 48-inch schmidt plates, are expected to have a symbolic velocity of recession  $V_s = c \times \Delta\lambda/\lambda$  of approximately 150,000 km/sec. Each cluster is photographed with the 200-inch three times on 103a-O plates (exposures 3, 9, 27 minutes) and three times on 103a-D behind GG 11 filter (exposures 5, 15, 45 minutes). The main results obtained so far are: (1) The total population of these clusters in the first three magnitude ranges  $m_{\max}$  to  $m_{\max}+3$  is very closely the same regardless of the value of  $m_{\max}$ . (2) The structural indices and the distribution indices are closely the same for clusters at all distances. (3) The segregation of bright and of faint galaxies within the clusters is the same regardless of their distance. (4) The total population of the clusters in a first approximation is proportional to  $\gamma - \gamma_0$ , where  $\gamma$  is the angular diameter, and  $\gamma_0$  is a constant. (5) The number  $n_c$  of member galaxies per limiting square degree in the center of the clusters



in a first approximation is inversely proportional to the angular diameter  $\gamma$ , provided that no interstellar or intergalactic absorption interferes. These results allow a distance determination from counts of cluster galaxies alone, the distance being inversely proportional to the apparent structural index.

During the summer of 1956, Abell completed the compilation of a catalogue of 2712 rich clusters of galaxies discovered on the National Geographic Society-Palomar Observatory Sky Survey. From these he selected a homogeneous sample of 1682 clusters each of which contained at least 50 members within 2 magnitudes of the third brightest member. A statistical analysis of this sample indicates: (1) The distribution function of clusters according to richness increases rapidly as the population per cluster decreases. (2) The data allow no significant decision that the spatial density of cluster centers varies with distance. (3) Galactic obscuration of the order of a few tenths of a magnitude (photo-red) exists at high northern galactic latitudes around longitude  $300^\circ$ . (4) There is a highly significant nonrandom distribution of clusters in direction in the sky, both when clusters at all distances and when clusters at various distances are considered.

In conjunction with the new catalogue of galaxies, a catalogue of the richest clusters of galaxies is being constructed by Zwicky, including the 1950 positions of the centers, the populations, characters, apparent diameters, and estimated distances of all clusters. Contours of all clusters, that is isopleths for which the population per square degree is about twice that found in the surrounding field, are plotted on the same charts containing the galaxies of the catalogue mentioned.

Clusters of galaxies are the only objects that can be used for distance indicators or for the study of such problems as the velocity-distance relation out to the extreme range of the large telescopes. Because of the importance of locating as distant clus-

ters as possible, Baum made a series of experimental exposures with the 48-inch telescope in a study of the most effective techniques for locating clusters at the extreme limit of the instrument. Experiments were made with Eastman 103-U plates in combination with a Chance OR-1 filter to obtain a range of response from roughly 6500 to 7500 Å. This range was selected for two reasons: it lies within a spectral region unusually free of radiation from the night airglow; it particularly favors galaxies of the type sought, namely, those having redshifts equivalent to about half the velocity of light. Although the advantage over the red-sensitive plates ordinarily used amounts to only a fraction of a magnitude in threshold detection, about a dozen suspected clusters were observed in this manner, and four of them were rephotographed with the 200-inch telescope. Since all these clusters were found to have relatively small membership, it will be worth some further searching, possibly with the additional aid of finer-grained emulsions.

#### *Velocities and Distances of Galaxies*

During the past three years, observations have been made by Humason at the prime focus of the 200-inch for the purpose of obtaining larger redshifts than had heretofore been measured. The results of this investigation have been negative in that it has not been possible to identify known spectral features with the certainty required for the measurement of redshifts. Inability to obtain usable spectra is due to the following reasons. As the present sunspot maximum develops, intensity of the airglow spectrum has increased to the point where it almost obliterates the spectrum of a faint galaxy. Magnitudes of the brightest members in the clusters observed are fainter than 20.0. The spectra are extremely narrow because the diameters of distant galaxies are small. Redshifts of very distant clusters are large enough to displace such well known features as the



G band, H and K, and the emission at  $\lambda 3727$ , beyond the long-wavelength limit of fast blue emulsions. All this has necessitated the use of slower panchromatic emulsions, which increases the exposure times by a factor of 10 or more.

The negative results obtained for this particular investigation are reported on here both because the observations have consumed much valuable 200-inch time and because the data given below will locate and identify the clusters for other observers who may intend to continue this type of research. All the clusters listed in table 3 were first found on plates of the National Geographic Society-Palomar Observatory Sky Survey and later rephotographed by Sandage with the 200-inch.

TABLE 3. Clusters

(1950)			Decl.	Possible Redshift
R.A.				
0 <sup>h</sup>	24 <sup>m</sup>	0 <sup>s</sup>	+ 16° 53'	103,000 or 167,000
10	44	11	+ 9 20	63,000
13	32	10	+ 28 28	74,000
14	47	33	+ 26 22	118,000

Co-ordinates are for 1950, and refer to the center of each cluster. In the last column appear possible values of the red displacements. It should be stated that they are unmeasured, and their reality is uncertain. They were obtained from one or more spectral features which seemed to be dimly visible but could not be positively identified.

Two uncertain displacements are given for the first cluster. If either is real it is most probably the smaller value. The most certain value is that for the second cluster in the list, +63,000 km/sec.

During the report year, Baum has continued the photoelectric program for determining both the redshifts and the magnitudes of remote galaxies by multicolor photometry. It consists in measuring their relative luminosities in a number of different colors ranging from 3800 Å in the ultraviolet to 10,000 Å in the infrared. The data for each object yield a curve of radiated energy  $E$  as a function of wavelength

$\lambda$ . For a galaxy that is shifted to the red, the whole  $E(\lambda)$  curve is displaced toward longer wavelengths, and the amount of the displacement yields the "velocity" of recession. The result is the same as a spectrographic measurement of the amount by which individual spectrum lines are displaced, but multicolor photometry has the advantage of being able to reach galaxies considerably fainter and more distant than those within spectrographic reach. The  $E(\lambda)$  curves also provide bolometric magnitudes directly without the need for K conditions.

This multicolor photoelectric procedure rests on two conditions: the  $E(\lambda)$  curves must be on a true scale of energy per unit wavelength; the galaxies of one cluster being compared with those of another cluster must be intrinsically similar, that is the differences between their  $E(\lambda)$  curves must be due largely to their redshifts and not to other effects. Although this second condition cannot be guaranteed for unlimited distances, neither Whitford nor Baum now finds any clear evidence for other reddening effects within the range of distances reachable spectroscopically. There is, however, the possibility that effects due to evolution or obscuration may influence the magnitudes more strongly than the  $E(\lambda)$  redshifts. This possibility can be checked by comparing magnitudes with apparent angular diameters estimated from photoelectrically measured brightness profiles, and efforts were continued during the report year to obtain the profile observations required.

The photoelectric redshift-magnitude data are being accumulated as rapidly as telescope time permits, and some interesting results are beginning to emerge. Observations obtained thus far extend from the near-by cluster of galaxies in Virgo (about 10 megaparsecs distant) to one of the remotest clusters of galaxies detectable on the 48-inch Sky Survey plates. The  $E(\lambda)$  redshift of this latter cluster was found to be of the order of 120,000 km/sec (or  $0.4c$ ), which is roughly twice that of the present spectroscopic limit.

## RADIO SOURCES

### *Identification of Radio Sources*

In a sample area between  $0^h$  and  $6^h$ ,  $-9^\circ$  to  $+3^\circ$ , a detailed study is now being carried out by Minkowski of those objects that may be of interest in connection with the problems of the identification of radio sources. For this area the records and results by Mills and the results of the Cambridge Survey were compared with the prints of the National Geographic Society-Palomar Observatory Sky Survey during Minkowski's stay at the Division of Radio-physics of the Commonwealth Scientific and Research Organization, Sydney, Australia. The aim of the study is not primarily to identify a few more radio sources, but to obtain a clear picture of the limitations to which attempts at identification are subjected, in particular for extragalactic sources. Since the intrinsic strength of sources has a very large dispersion, the situation differs for strong and for weak sources. Intrinsically very strong extragalactic sources are expected to be optically of low apparent brightness, since Cygnus A, the prototype of this class, is already of 18th magnitude. The accuracy of the positions in the present radio surveys does not permit the identification of objects fainter than this, and additional identifications of intrinsically strong radio sources will be possible only when more precise positions become available. Intrinsically weak extragalactic sources may be optically relatively bright, such as NGC 5128 or NGC 1275. Such objects can easily be photographed and recognized as peculiar at distances at which they are beyond the sensitivity limit of present radio telescopes. At present, only radio sources of intermediate intrinsic intensity can be expected to be identifiable. The results obtained in the still-incomplete study show already that at the present stage of technical development not more than a small percentage of all sources can be identified with galaxies. Since the available evidence suggests strongly that stars are not radio sources, it seems prob-

able that most radio sources are distant galaxies fainter than 18th magnitude.

Two regions, previously found by Matthews to have an excess of 21-cm radiation, have been identified by him with features appearing on the 48-inch survey. The first of these regions is in the OB aggregate I Camelopardalis, where the schmidt plates show a region of very high absorption about  $4^\circ$  across with a few spots of weak  $H\alpha$  emission around the edges. About three-quarters of the stars in the OB aggregate are located less than  $1.5^\circ$  from the absorbing region. Their proximity, together with the observed 21-cm radial velocity of the feature, suggest that the distance to the absorbing cloud is 1 kiloparsec.

The 21-cm observations show that the feature is  $6^\circ$  across, which gives a diameter of 105 parsecs. The total mass of neutral hydrogen in the region is  $7.8 \times 10^4$  times the mass of the sun, giving a mean density of  $5.5 \text{ H atoms/cm}^3$ . This mass is the same as the mass of neutral hydrogen in the Orion region given by T. K. Menon. The 21-cm profiles show that the expansion of the region, if any, is less than 3 km/sec. Photoelectric measures of selected stars in the OB aggregate and in an open cluster situated within the area of the absorbing cloud are in progress.

The second region under study is at galactic longitude  $100^\circ$ , latitude  $+11^\circ$ . The 48-inch survey shows some absorbing clouds to be present. Deep  $H\alpha$  photographs, taken with the 48-inch schmidt, show the presence of faint  $H\alpha$  emission in the same region that has the excess 21-cm radiation. No star or stars, down to the 10th magnitude, are known either within the region or near by which could provide the ionizing radiation to produce the  $H\alpha$  emission. Thus collisional excitation is probably present. Further observations are in progress, and calculations will be made to check this hypothesis.

The 32-foot-diameter radio telescope on Palomar Mountain is being used by Mat-



thews to make a 21-cm survey of the Milky Way region between galactic longitudes  $190^\circ$  and  $250^\circ$ . The coverage in galactic latitude extends up to  $16^\circ$  from the plane of the Galaxy. Preliminary results on the distribution of neutral hydrogen in the galactic plane show a good agreement with the Dutch and Australian results. The hydrogen belonging to the Orion arms shows the presence of systematic velocities in varying amounts up to 10 km/sec between longitudes  $200^\circ$  and  $240^\circ$ . A survey of the hydrogen distribution near selected galactic radio sources is also in progress.

In order to provide independent evidence on the distance of the Cassiopeia radio source, a search for faint B stars in that field was made by Luis Münch on  $H\alpha$  plates taken with the Tonantzintla prismatic camera and a red filter. Three stars tentatively classified as B stars were found at distances of 1.6, 2.2, and 5.3 from the

center of the source as given by Baade and Minkowski. Photoelectric colors in the UBV system of these three stars, determined by Luis and Guido Münch at the 60-inch telescope, have shown that indeed these stars are of type B, with color excesses  $E(B-V)$  around 1.0 mag. Although no structure in the interstellar lines of one of these stars has been observed, the measured K-line radial velocity suggests that the star is in the Perseus spiral arm. Since the reddening of the source estimated by Baade and Minkowski is larger than that of this star, it would seem that the distance of the source is larger than 2 kiloparsecs, in agreement with the distance determination from the 21-cm absorption lines. Spectroscopic observations are planned for the two other fainter stars, which are at closer distance to the center of the source, in order to make the evidence somewhat stronger.

## INSTRUMENTATION

The idea of the pneumatic mirror, formed by stretching a thin solid film over an optically finished ring, and aluminizing it, has been developed by Horace W. Babcock, and experimental work on such a mirror up to 10 inches in size is being carried out in the laboratory. Such a mirror, when provided with a backing plate a short distance behind the film, has pneumatic stiffness against fluctuations in atmospheric pressure. It also has the interesting property that, in the flat form, its optical figure is independent of the temperature of the film. The film can readily be cooled from behind, as is desirable for mirrors of solar telescopes. In principle, if a differential pressure is applied to the opposite sides of a uniform film, a paraboloidal figure is obtained.

Swanson has ruled 12 large gratings dur-

ing the year, and has devoted much time to tests and improvements of various parts of the ruling machine. The most notable accomplishment was the ruling of an excellent grating having the exceptional width of 10 inches and a groove length of 6 inches. It is not only the largest high-precision grating ruled here, but it is fully the equal in quality with any of the earlier and smaller gratings. Its resolving power, though not yet quantitatively measured, is distinctly the best yet seen, showing the anticipated improvement over 8-inch gratings. This accomplishment proves that the ruling engine is completely successful up to the limits of its dimensional capacity, and that a ruling diamond can, on occasion, produce more than 14 miles of uniform precision grooves on a single plate without appreciable wear.

## GUEST INVESTIGATORS

The Observatories have invited a number of guest investigators to make use of such observational facilities as were not

required by the programs of the regular staff. The following studies have been carried out by these investigators.



Dr. George O. Abell, of the Department of Astronomy at the University of California at Los Angeles, investigated the bright end of the luminosity function of galaxies in rich clusters with the 48-inch schmidt telescope. For the investigation about 30 clusters have been chosen from the catalogue of clusters compiled from the National Geographic Society-Palomar Observatory Sky Survey photographs. Magnitudes of galaxies (to within about 0.1 mag.) are obtained by extrafocal photographic photometry, extrafocal images of galaxies being compared with extrafocal images of stars which are calibrated with photographic transfers from Selected Areas. In addition to the luminosity function, information is obtained about the spatial distribution within clusters of galaxies of various luminosities and (presumably) masses. The observational phase of the program is now about 30 per cent complete.

Observations of 26 planetary nebulae have been secured with a photoelectric scanner attached to the 60- and 100-inch telescopes by Dr. Lawrence H. Aller and Dr. William Liller, of the University of Michigan. Dispersion is provided by a 600 line/mm reflection grating, with identical  $f/5$  Newtonian systems used as the collimating and focusing units. The spectrum is scanned with either a blue-sensitive or a red- and infrared-sensitive photomultiplier permitting a study of the spectrum from  $\lambda 3200$  to  $\lambda 12,000$  Å. The total nebular brightnesses were measured photoelectrically at Mount Wilson in 1954. By combining both the direct and spectral observations with previously obtained photographic data for the fainter lines and the isophotic contours, Drs. Aller and Liller expect to obtain a better assessment of stratification effects together with improved ionic concentrations and electron temperatures. The variability of the spectrum of IC 4997 has been established.

Dr. Dinsmore Alter, of the Griffith Observatory, has continued his photographic observations of the moon with the 60-inch

telescope on 11 nights of above-average seeing. Photographs were taken in blue-violet and in infrared light, special attention being given to the regions of Ptolemaeus, Alphonsus, Arzachel, and Atlas under a low setting sun in an attempt to obtain evidence for scattering by traces of escaping gas.

Dr. James Cuffey, of Indiana University, obtained photoelectric measures of colors and magnitudes of faint stars in the globular clusters M 53 and NGC 5466 with the 60- and 100-inch reflectors in April 1957. The photoelectric standards are being used to calibrate photographic observations of the color-magnitude relations as faint as the 19th magnitude.

The solar furnace located on the roof of Robinson Hall at the California Institute has been reconditioned and used for high-temperature materials studies by the Stanford Research Institute in collaboration with Dr. Paul Duwez, of the Department of Engineering of the California Institute. The great advantage of a solar furnace in materials research is the high concentration of radiant heat over a small area ( $\frac{1}{2}$  inch in diameter in the present apparatus). Because of this highly localized heat flux, melting of a portion of a solid sample may be achieved so that the material under study serves as its own crucible. Refractory substances that react with any known crucible can therefore be melted without being contaminated. Compounds involving two of the most refractory oxides, namely, thorium oxide ( $3200^{\circ}\text{C} \pm 100^{\circ}\text{C}$ ) and zirconium oxide ( $2750^{\circ}\text{C}$ ), were successfully melted in the furnace. The structure of these compounds was investigated by X-ray diffraction methods, and the results of these studies led to a better understanding of one of the most heat-resisting solid materials of engineering interest. At present, the solar furnace is being used for the study of compounds of uranium dioxide and zirconium dioxide, which are of great potential interest in the development of fuel elements for high-temperature nuclear reactors.

Dr. Carlos Jaschek, of the Observatorio Astronómico de la Universidad Nacional de La Plata, obtained an extensive series of spectra of metallic-line stars in order to ascertain the existence of families among these stars in analogy to the existing families among peculiar stars. Spectra of 35 stars of this type were taken with the 60-inch telescope at a dispersion of 21 Å/mm. Five spectra of peculiar A-type stars were taken with the 100-inch coudé spectrograph for a detailed analysis of the atmospheres of these objects.

Spectrograms of  $\tau$  Coronae Borealis at 10 Å/mm were obtained by Dr. Robert P. Kraft, of Indiana University, in an attempt to classify the motions of the components of this binary star. Dr. Kraft also observed the spectra of several of the fainter members of the open cluster NGC 6664 in order to obtain radial velocities and spectral types.

Spectrograms of 39 long-period variable stars with types M0 to M5 were taken with the 4-inch camera on the X spectrograph of the 60-inch by Dr. Philip C. Keenan, of the Perkins Observatory. This group of variables was selected because they are made up, at least in part, of Population II stars which include some with luminosity high enough to make them valuable as distance indicators for moderately distant stellar systems. The spectra give evidence of a considerable spread in luminosity within the group. RT Cygni and Z Ophiuchi, for example, are probably supergiants at least as bright as  $\alpha$  Orionis, while the least luminous stars in the group are more comparable to ordinary red giants. More data will be needed to fix the scale of absolute magnitudes of these variables, but one means of doing that was found when two of the stars, R Trianguli and X Monocerotis, were photographed in the red region with the 100-inch coudé spectrograph. The large velocity shifts in these spectra made it possible to see interstellar components of the sodium D line, and these components turned out to be quite strong in both stars. When more such

cases are found it will be possible to apply the usual methods, involving intensities and displacements of the interstellar lines, to derive mean luminosities.

Studies of the air currents and "seeing" at the 60-foot solar tower have been carried out by Dr. R. B. Leighton, of the Physics Department of the California Institute. These investigations have shown that it is possible to reduce the effects of local thermal air currents by appropriate treatment of exposed surfaces near the optical path through the tower, and there appears to be considerable hope of prolonging the period of early-morning good seeing to an hour or more.

Three hundred feet of 16-mm Kodachrome film were exposed by Dr. Leighton in a photographic study of Mars during the period August 6 to October 15, 1956. Many photographs of excellent quality were obtained. Study of these photographs is still in progress.

Visual observations of double stars were made with the 60-inch reflector at the Cassegrain focus on portions of the nights of August 16 to 19 and all night at the Newtonian focus on August 20–21 by Dr. William Markowitz, of the U. S. Naval Observatory. The seeing was good enough on all nights to permit measurement of close doubles. The Airy disks were seen on all nights. The powers generally used were 1300 at the Cassegrain and, with a Barlow lens, 2000 at the Newtonian. Images obtained in the second manner were superior to those obtained at the Cassegrain without a Barlow lens. Thirty-one measures of 23 pairs were made. Most of the pairs had separations from 0''.09 to 0''.19. L726-8 was measured. The results of these tests indicate that the 60-inch may be used to a limited extent for the measurement of very close pairs and in searching for duplicity in suspected faint stars.

The co-operative program with the McMath-Hulbert Observatory was continued throughout the year as in the past 6 years. The observer for the project stationed on Mount Wilson was Mr. Thomas K. Jones.



Dr. Robert R. McMath spent a number of days in February inspecting the Snow telescope instrument and reviewing the various aspects of the program. The program for the Snow telescope for the current report year has been the following: (1) continuation of the systematic observation of the infrared helium line (10830 Å) at the limbs of the sun and in the plage regions; (2) systematic observation of the central structure of the K line (3934 Å) at the limbs of the sun and in plage regions; (3) observations of a number of selected lines in the region 7500 to 12000 Å that should be good indicators of physical conditions on the sun; (4) observations of a number of lines that are badly blended with water-vapor lines (such as H $\alpha$ ) for comparison with McMath-Hulbert tracings made under conditions of high water-vapor content in the earth's atmosphere; (5) continuation of infrared-sunspot tracings as suitable spots have developed on the solar disk; (6) repetition of tracings for wavelength measurement in the 3 to 5  $\mu$  region with the Lallemand PbTe cell.

Observations were made on 120 days; 760 tracings were produced. These totals are considerably less than in previous years because instrumental troubles with the spectrometer made necessary an almost complete overhaul with considerable rebuilding of worn parts of the mechanism.

The observations on dry days on Mount Wilson are proving to be very valuable for eliminating the effect of water-vapor lines from tracings made with higher dispersion at the McMath-Hulbert Observatory with the McMath-Hulbert vacuum spectrograph.

The programs of observation of the Ca<sup>+</sup> K lines and the 10830 Å line of helium are now approaching a critical stage as the sunspot activity increases. The continuation of the recording throughout the present maximum will complete the observational history of the variation of these lines throughout a sunspot cycle.

With the present Babcock gratings and the reconditioned spectrometer, measure-

ments of improved precision are being made in the 3 to 5  $\mu$  region. The new measurements are being made on tracings obtained with a Lallemand PbTe cell, whose increased sensitivity with respect to earlier cells also makes increased accuracy possible.

Spectroscopic observations of the eclipsing systems RZ Scuti and U Cephei were made by Dr. D. H. McNamara, of Brigham Young University. The H $\alpha$  line in the spectrum of RZ Sct is of great interest. The absorption line is bordered by emission that is particularly pronounced during eclipse but is also faintly present outside of eclipse. The H $\alpha$  absorption line undergoes a change in width as well as in intensity during the primary eclipse. When the brighter component emerges from eclipse, the line is 1.3 times wider than at other phases. Asymmetries can be detected in the H $\alpha$  line several days before and after eclipse. For U Cephei a new velocity curve has been derived from plates taken with the 60-inch telescope. Of particular interest is the discovery of a new distortion in the velocity curve in the form of a rapid rise and decline in the velocity of the order of 25 km/sec occurring near phase 0.8 day.

Dr. W. W. Morgan, of the Yerkes Observatory, continued his work on the spectral classification of galaxies which he had started earlier in collaboration with Dr. N. U. Mayall, of the Lick Observatory. While at the Mount Wilson and Palomar Observatories he studied the files of spectrograms obtained by Humason and the direct photographs of Hubble, Humason, Sandage, and Baade. A period of five nights with the 100-inch nebular spectrograph was used to obtain new spectra of several elliptical galaxies; these made possible the determination of spectral types of the systems in the ultraviolet. A discussion of the above material, together with spectrograms obtained by Mayall at Lick, has led to the following conclusions: The great majority of the galaxies can be classified by their spectra into about five classes; if these are denoted by the spectral types



as determined in the region of 4000 Å, the spectroscopic groups range from A to K. There is a marked correlation between the degree of central condensation and the spectral type of the system as a whole; those of type A show little or no central condensation; on the other hand, those of type K consist of the ellipticals and the Sa and Sb systems having pronounced central condensations.

From a study of objective-prism plates taken with the schmidt camera of the Tonantzintla Observatory a number of hitherto unclassified OB stars in longitude  $82^\circ$  to  $92^\circ$  have been selected in order to determine to what extent the OB associations I, II, and III Cassiopeiae are separated from one another in space. Fifty stars have been observed with the 4-inch camera of the X spectrograph of the 60-inch telescope, and their spectral types on the Yerkes system have been determined by Luis Münch, of the Tonantzintla Observatory. It is planned to reobserve spectroscopically as many of these as possible to derive radial velocities. The photoelectric colors and magnitudes of these stars are also being measured to obtain spectroscopic parallaxes.

A number of stars classified on objective-prism plates as peculiar A stars have been observed with the X spectrograph in order to relate the criteria of peculiarity with those established in the Yerkes system. The stars BD  $-4^\circ 1644$ ,  $-1^\circ 1414$ , and  $-3^\circ 1665$  were also found to be spectrum variables, and their variations are being studied. A period of 4.1 days has been derived for the variation of Ca II K in the spectrum of BD  $+46^\circ 1913$ . Photoelectric observations of these same objects are being carried out with the 20-inch telescope of Palomar Mountain.

The radial velocities of the most distant early-type stars known in galactic longitudes  $325^\circ$  to  $10^\circ$  are being determined from plates taken with the 8-inch camera of the X spectrograph by Luis Münch in collaboration with Guido Münch. The purpose of this program is to reanalyze the problem of the discrepancy between

the rotational velocity of the inner parts of the galactic system as determined from stellar radial velocities and 21-cm-line radio observations.

Dr. L. Plaut, of the Kapteyn Astronomical Observatory, Groningen, has completed the observational part of his investigation of the large-scale structures of the halo of the galactic system. Nearly 500 plates were taken with the 48-inch schmidt camera. They are now being searched in Groningen for variable stars. Four areas are being investigated, centered at the following galactic co-ordinates:  $l=327^\circ.5$ ,  $b=+28^\circ$ ;  $l=327^\circ.5$ ,  $b=-12^\circ$ ;  $l=331^\circ.0$ ,  $b=+12^\circ$ ; and  $l=147^\circ.5$ ,  $b=+15^\circ$ .

Dr. Daniel M. Popper, of the University of California at Los Angeles, has continued his investigation of the orbits and masses of eclipsing binaries. He has recently re-discussed the eclipsing binary Z Vulpeculae on the basis of new photoelectric observations at Palomar as well as of spectroscopic observations. The principal interest in Z Vul is that it contains an A-type star of luminosity class III, the first star of this type to have reliable determinations of mass and radius. During the current report year, one star, V 477 Cygni, has been added to the list of systems in need of revision of masses and radii, and two stars, RY Persei and RS Vulpeculae, have been added to the list of systems with components above the main sequence for which masses will eventually be determined. Observations on RX Herculis and RS Canum Venaticorum have been nearly completed. The mass of the former system is found to be 30 per cent larger, that of the latter about 40 per cent smaller, than the previously published values. Clearly, higher dispersion than heretofore must be applied to many systems. Fair progress has been made on the difficult problem of determining the masses of  $\zeta$  Aurigae from spectrograms. Although final results are not available, the published value for the mass of the cool supergiant appears to need serious revision downward.

Drs. Otto Struve and Jorge Sahade, of

the University of California at Berkeley, have continued their spectroscopic observations throughout the year. Among the results obtained from these observations are:

1. Emission lines (strong at  $H\alpha$ ) were discovered in the spectrum of Algol. The emission is observed at quadratures, and is different in character from the emission previously observed in eclipsing systems.

2.  $H\alpha$  emission was also discovered in  $\beta$  Cephei, U Coronae Borealis, and HD 47129.

3. Individual cycles were found to differ in velocity amplitude in  $\delta$  Delphini, the total range varying from 1–2 km/sec to 4–5 km/sec.

4. Extensive studies were made of the spectrum of  $\epsilon$  Aurigae, which emerged from its last eclipse in May 1957. All the radial velocities have been measured, and the results indicate large changes of an irregular nature which are not caused by the geometrical properties of the double-star system. A spectrophotometric study of the best plates during and outside of eclipse, made in Berkeley with the cooperation of Dr. Margherita Hack, has indicated that the general character of the eclipsing body is very much like that previously described by Struve. The invisible nucleus in the center of the large eclipsing shell may be a subluminous B-type star.

5. The spectrograms of  $\beta$  Lyrae have been enlarged and assembled in the form of an atlas. The radial velocities of  $\beta$  Lyrae have been used to determine the orbital elements with the help of the IBM 701 in Berkeley. The precision of this orbit is of a high order, and will form the basis for future studies of the perturbations in the system.

6. Observations have been made of the following additional stars: W Serpentis, AZ Cassiopeiae,  $\delta$  Capricorni, 12 Lacertae, and 17 Leporis.

Professor A. Unsöld, of the University of Kiel, obtained a series of spectrograms for a detailed study of the atmospheres of several stellar types. They included spectra of medium-type subdwarfs, red and infra-

red spectra of normal bright stars, spectra of later-type main-sequence stars, and spectra of M dwarfs of emission and nonemission types.

Photoelectric observations of the short-period eclipsing binary Nova DQ Herculis (1934) were obtained on five nights during July and August with the 100-inch reflector by Dr. Merle Walker, of the Warner and Swasey Observatory. From these observations, the following improved elements have been derived:

$$\text{Min} = \text{Hel JD } 2434954.94475 + 0^d 19362060\text{E}$$

An analysis shows that the *form* of the eclipse curve has changed since 1954, presumably owing to the presence of gas streams or of detached material in the system. It is not possible to obtain reliable photometric elements from the 1956 observations, and the change in the light-curve throws considerable doubt on the validity of the photometric elements derived from the 1954 observations. The observations were made in yellow light to avoid the effect of the nebulosity surrounding the system. A more detailed study of the 1-minute periodic variations has been made from the 1956 observations. It has been possible to derive the period of these variations over a 2-day interval. The elements are

$$\text{Max} = \text{Hel JD } 2435660.7113 + 0^d 000822528\text{E}$$

for the observations made in July; for those in August, the epoch is

$$\text{Hel JD } 2435695.7499$$

The change in the visibility and amplitude of these oscillations with the phase in the 4-hour period suggests that they originate on the hemisphere of the Nova facing the secondary star. There are complications, however.

Simultaneous photometric and spectroscopic observations were obtained of AE Aquarii by Dr. Walker in collaboration with Deutsch. The photometric observations, made with the 60-inch reflector, were used to direct the spectroscopic observer at



the 100-inch to place the star in one of two regions of the slit, depending upon whether it was momentarily bright or faint. In this way two spectra were eventually built up, corresponding to the maxima and minima of the rapid variations in light displayed by the star. In the red, the equivalent widths of all absorption and emission lines, including  $H\alpha$ , appear to be the same in the two spectra. In the photographic region, the continuous spectrum is much bluer at maximum light, and all emission and absorption lines have decidedly smaller equivalent widths, including the Balmer lines.

The infrared spectra of bright stars were examined by Dr. A. E. Whitford, of Washburn Observatory, University of Wisconsin, using a germanium photodiode at the exit slit of the scanning spectrograph. The new detector shows considerable promise in the range from 10000 to 16000 Å, having a detection limit about 100 times better

than lead sulfide photoconductive cells. An exit slit 20 Å wide gave a resolving power 6 to 10 times better than had previously been achieved on stars beyond the photographic limit. This resolution was adequate to show the stronger atomic absorption lines, such as the Paschen series of hydrogen, and molecular absorption bands. The detector sensitivity would permit going to a resolution of 2 to 4 Å, which would bring out subordinate metallic lines; a different optical arrangement would be necessary, however.

Over the more conventional photomultiplier range of 3400 to 10000 Å, Dr. Whitford scanned the spectrum of NGC 4374, one of the bright elliptical galaxies in the Virgo cluster, in order to compare its spectral energy distribution with that previously found for M 32 by Code. Selected pairs of reddened and unreddened stars were also observed with the scanner as a test of the law of reddening.

## STAFF AND ORGANIZATION

Dr. Milton L. Humason and Dr. Seth B. Nicholson retired from the staff of the Observatories on June 30, 1957. Dr. Humason joined the Mount Wilson Observatory in 1917, first as janitor and then as night assistant. In the latter position he displayed such skill as an observer that he was made a member of the Staff of Investigators in 1922. He first assisted Dr. Merrill in a survey for early-type stars with bright hydrogen lines. Later he collaborated with Drs. Adams and Joy and Miss Brayton in their very extensive study of stellar absolute magnitudes and spectroscopic parallaxes, which resulted in the publication of the magnitudes and parallaxes of 4179 stars in 1935.

In the course of these studies Humason developed a very unusual proficiency in the photography of spectra of very faint objects. After the discovery by Hubble in the 1920's of the major role played by the galaxies in the structure of the universe, Humason turned his attention to the spectra of these objects and soon accumulated

spectra of a substantial number of these galaxies spread over a wide range of distances. A study of the relationship between the velocities as measured on these spectrograms and the distances of these galaxies led Hubble to the concept of the expanding universe. For the next quarter century Humason devoted most of his attention to this problem. The introduction of extremely fast photographic plates, the development of new and very rapid spectrographs, and the completion of the 200-inch Hale telescope enabled Humason to push his observations to fainter and fainter and therefore more and more distant galaxies. These techniques now permit photographing the spectra of galaxies far too faint to be seen visually with the telescope used to collect the light. Humason therefore had to develop elaborate offset procedures that ensure locating invisible images accurately on the slit of the spectrograph and holding them there during long exposures. His studies culminated in the publication in 1956, in collaboration with



Dr. N. Mayall, of the Lick Observatory, and with Dr. Sandage, of the velocities of over 900 galaxies. Some of these velocities are as high as one-fifth that of light.

In 1948 Dr. Humason was appointed Secretary of the Observatories. As Secretary he has ably handled the correspondence and public relations as well as many of the other administrative problems of the Observatories.

Dr. Nicholson joined the staff of the Mount Wilson Observatory in 1915. During the first few years he investigated the orbits of several of Jupiter's satellites, the ninth of which he had discovered at Lick Observatory in 1914. In collaboration with Dr. Pettit he developed a very sensitive vacuum thermocouple. This they used to measure the total radiation and surface temperature of stars, the planets, and the moon. Their data on the cool long-period variables were of special value. Studies of the rates of cooling of the moon's surface during an eclipse gave a measure of the thermal conductivity of the surface rocks and provided information on their physical characteristics. In the late 1930's and again in the early 1950's Dr. Nicholson returned to the observation of Jupiter's satellites, discovering the tenth, eleventh, and twelfth of these objects and determining the positions necessary to fix their orbits.

Throughout Dr. Nicholson's 42 years at the Observatories a large part of his efforts has been devoted to solar observations, at first in collaboration with Dr. Hale. He has developed an extraordinarily detailed knowledge of the complex phenomena of the sun's visible surface. He has supervised the systematic collection of data on sunspots, including the polarity and strength of their magnetic fields. In collaboration with Dr. Oliver Wulf, of the U. S. Weather Bureau, he has made detailed investigations of the correlation between solar and terrestrial phenomena.

Mr. Edgar C. Nichols retired as Chief Designer and Superintendent of the Instrument Shop on February 28, 1957, after 46 years of service at the Observatories. Many

of the instruments on Mount Wilson owe much of their efficiency and ease of operation to Mr. Nichols' skill as a designer.

Dr. Horace W. Babcock was appointed Assistant Director of the Observatories effective January 1, 1957. Dr. Arthur D. Code became a member of the Staff of the Observatories on September 1, 1956, and Dr. Halton C. Arp on July 1, 1957.

### *Research Division*

#### *Staff Members*

Halton C. Arp  
Walter Baade  
Horace W. Babcock, *Assistant Director*  
William A. Baum  
Ira S. Bowen, *Director*  
Arthur D. Code  
Armin J. Deutsch  
Jesse L. Greenstein  
Milton L. Humason, *Secretary of the Observatory*<sup>1</sup>  
Rudolph L. Minkowski  
Guido Münch  
Seth B. Nicholson<sup>1</sup>  
Donald E. Osterbrock  
Robert S. Richardson  
Allan R. Sandage  
Olin C. Wilson  
Fritz Zwicky

#### *Carnegie Research Fellows*

Geoffrey R. Burbidge  
Thomas A. Matthews  
Maarten Schmidt

#### *Research Assistants*

Sylvia Burd  
Mary F. Coffeen  
Thomas A. Cragg  
Dorothy S. Deutsch  
Edith Flather  
Emil Herzog  
Joseph O. Hickox  
A. Louise Lowen  
Mildred Matthews  
Carol Nordquist  
Henrietta H. Swope

#### *Student Observers*

George O. Abell  
Walter K. Bonsack  
Jacques Feige

<sup>1</sup> Retired June 30, 1957.

William G. Tifft  
Dale Vrabec  
George Wallerstein

*Editor and Librarian*

Alexander Pogo

*Photographer*

William C. Miller

*Instrument Design and Construction*

Lawrence E. Blakeé, Electronic Technician  
Floyd E. Day, Optician  
Kenneth E. DeHuff, Machinist  
Robert D. Georgen, Machinist  
Don O. Hendrix, Superintendent, Optical Shop  
Melvin W. Johnson, Optician  
Edgar C. Nichols, Chief Designer, and Superintendent, Instrument Shop<sup>2</sup>  
Bruce Rule, Project Engineer  
Oscar Swanson, Instrument Maker

*Maintenance and Operation*

*Mount Wilson Observatory and Offices*

Audrey A. Acrea, Stewardess  
Paul F. Barnhart, Truck Driver  
Ashel N. Beebe, Superintendent of Construction

<sup>2</sup> Retired February 28, 1957.

Wilma J. Berkebile, Secretary  
Ernest V. Cherry, Janitor  
Hugh T. Couch, Carpenter  
Eugene L. Hancock, Night Assistant  
Emerson W. Hartong, Truck Driver  
Anne McConnell, Administrative Assistant  
Leah M. Mutschler, Stenographer and Telephone Operator  
Bula H. Nation, Stewardess  
Alfred H. Olmstead, Night Assistant  
Arnold T. Ratzlaff, Night Assistant  
Clyde Sanger, Gardener  
John E. Shirey, Janitor and Relief Engineer  
Benjamin B. Traxler, Superintendent

*Palomar Observatory and Robinson Laboratory*

Fred Anderson, Machinist  
Dorothea Davis, Secretary  
Eleanor G. Ellison, Secretary and Librarian  
Ferd Feryan, Mechanic  
Arlis Grant, Stewardess  
Leslie S. Grant, Relief Night Assistant and Mechanic  
Byron Hill, Superintendent  
Charles E. Kearns, Night Assistant  
Harley C. Marshall, Office Manager  
George W. Pettit, Janitor  
Robert E. Sears, Night Assistant  
William C. Van Hook, Electrician and Assistant Superintendent  
Gus Weber, Assistant Mechanic

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# COMMITTEE ON IMAGE TUBES FOR TELESCOPES

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During the past year the Committee has continued to encourage or foster several exploratory projects for the development of image tubes useful for increasing the range of telescopes. Several of these projects were outlined in the last report.

Although the secondary-emission image-multiplier tube still appears to offer the most attractive over-all solution to the image-tube problem, serious difficulties were encountered in attempts to make a pilot model.

Work both here and abroad has been carried on in an effort to make an image converter containing one or more stages of image intensification. Each stage consists of a very thin membrane with a phosphorescent screen on one side and a photocathode on the other. A gain of 10 or more per stage has been reported. These projects have thus far had very limited success, because of technical difficulties, dark emission, and loss of resolution.

During the past year no important advance, useful to astronomers, which has to do with the development of an electronic storage tube with a built-in electrical read-off system, has come to the attention of the Committee.

The main effort of the Committee was directed toward the development of thin-film image converters. Dr. W. Kent Ford, Jr., continued his important work, with the help of Committee funds, on the problem of making suitable thin metal films at the University of Virginia. The films transmit electrons but protect the photocathodes of the tubes from the molecules exuded from the emulsions of the nuclear-track plates which record the images of stars. The manufacturer mounted the films in place of the phosphorescent screens used in commercial tubes for viewing images directly. A special glass envelope was also attached to the rear end of the tube by the manufacturer to protect the

film from rupture as the tube was evacuated and sensitized. After the finished tube is installed in a special chamber mounted on the tail end of the telescope and a suitable vacuum around the glass cap is achieved, the cap is removed by cracking the glass along a circular groove by means of an electrically heated wire. The plate is next admitted through an air lock, and its emulsion is placed about 0.3 mm behind the thin film. Exposures are made by application of high voltages to the converter.

Dr. Ford's most successful films consist of an aluminum coating on Formvar and have a total thickness of about 0.15 of a wavelength of visible light. A large percentage of these films, mounted on Kovar rings, withstand baking for several hours at 300° C and are strong enough to be shipped through the mails.

One highly sensitive converter, having a thin film made by Ford and an S11 photocathode, was tested on the Naval Observatory's 40-inch telescope at Flagstaff, Arizona, by Drs. W. A. Baum and J. S. Hall. Unfortunately, the thin film was broken as the glass cap was removed just a few seconds before the first exposure was made. The contamination of the cathode was so rapid that no stellar image appeared. This rupture of the thin film was doubtless caused by gas pressure generated in the cap just as the glass was heated by the wire during the breakoff procedure. This phenomenon was not experienced during a test reported a year ago; in that test, however, the thin film contained pinholes, and only one useful exposure could be made.

In August 1957, after the close of this report period, a tube of low sensitivity was successfully used to record the images of several bright stars with the 40-inch telescope at the United States Naval Observatory.





# DEPARTMENT OF TERRESTRIAL MAGNETISM

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*Washington, District of Columbia*

MERLE A. TUVE, *Director*

RICHARD B. ROBERTS, *Acting Director, July–November 1956*

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*Magnetic station in Peru, 1957*

As part of its contribution to the International Geophysical Year, the Department of Terrestrial Magnetism is operating a line of four magnetic stations extending across the magnetic equator in Peru. The magnetic variometers normally recording at the observatory at Huancayo, now operated by the Instituto Geofísico de Huancayo and the Peruvian Government, serve as a fifth station in the network. With the help of the Instituto a preliminary survey was carried out in April and May 1957 for locating the four temporary IGY stations. Two aims of this work are to locate the narrow "electrojet" in the upper atmosphere, a belt of intense electric current which is normally strongest each noon, and to determine whether it is enhanced during magnetic storms. This electrojet current is part of the great system of currents in the high atmosphere which causes the daily variation of the earth's magnetic field. These variations and those during magnetic storms were noticed before A.D. 1700, and their cause has been sought for many years.



## INTRODUCTION

Basic research is usually described as simply the active expression of a curious or inquiring mind, whereas applied research is goal-directed and motivated by the hope of useful (and profitable) application.

There is a considerable area of overlap, however, as much of the activity of an investigator in "pure science" is goal-directed and practical to a high degree. This is because his deep interest in certain problems, or his "love for a subject," drives the investigator to find usable solutions to difficulties which he discovers must be overcome before he can examine or understand certain processes or conditions he has encountered in his studies. His actual work is nearly always "goal-directed," but its usefulness he measures in terms of the extent to which it enlarges his understanding and sharpens his concepts, and the profit lies in the corresponding enrichment of his own enjoyment of his subject. The subtle effect of his free-ranging curiosity and his corresponding willingness to be deflected from his immediate aims for a time, in order to inquire into some unexpected feature which he observes in the course of his systematic efforts, is another facet of difference between the man in basic research and his former classmate in the applied field. The principal distinction, however, probably lies in the different motivation of the individual and in the scale of values applied by the man and by his colleagues to the different types of contributions each man makes as time goes on.

This special kind of motivation of the individual investigator in "pure science" is of course strongly reflected in the character of the encouragement and support which the members of such a research staff are given. No fully effective research man is ever "dispassionate, detached, and disinterested"—on the contrary, he is passionately interested and very much immersed in his work. His intense emotional

investment simply makes greater demands on the homely qualities of honesty and integrity which are always so indispensable.

This report illustrates some of the "goal-directed" activities of a group of physicists who have been encouraged for some years to study and investigate those problems which have seemed to them of the most compelling interest, and each man has thus been invited to expand and deepen his love for his subject. The detailed problems range from hydrogen clouds among the stars to biochemical complexes, but this whole range of continually shared enthusiasms in the group finally adds up to a vigorous expression of the scope of "natural philosophy" and the creative use of experimental laboratory methods under intelligently analytical scrutiny.

In our studies of the radio waves arriving from outside the earth's atmosphere it is becoming increasingly apparent that optical identifications of the radio sources are necessary for progress in understanding just how large clouds of gas can radiate in the radio region. Even in studying that relatively near-by object, the sun, it seems that the position and intensity of the radio emission must be related to optically observed features on the sun before a satisfactory theory can be evolved. We have been led by this necessity to build, for scanning the sun, an antenna with angular resolution sufficient to separate two sources one-sixth of a solar diameter apart. This antenna takes the form of a line, 2000 feet long, of helical receiving elements, all connected in phase. With this antenna, and a receiver recording the power received at 335 mc/sec, the sun is slowly scanned by its own motion several times each day near local noon.

Observations of radio sources with test arrays at 400 mc/sec and with the 300 mc/sec helix array this year have provided interesting information on the possibility



of constructing relatively simple arrays capable of resolution of the order of one or two minutes of arc. Such accuracy is required to aid in the important problem of reliably identifying radio sources with optical objects.

In the last annual report, a discussion was given of some evidence suggesting that directed stresses occurring in the crust might have a profound influence on the magnetizations of various rocks. This possibility was examined by direct experiment this year with new equipment constructed specifically for the purpose; the results indicate that many conclusions that have been offered by various authors in recent scientific journals on the basis of rock magnetism data, relating to polar wandering, continental drift, secular variation and reversal of the earth's magnetic field, are subject to serious doubt because of the demonstrable sensitivity to stress of the magnetizations of many rocks.

By a combination of the rubidium-strontium, potassium-argon age methods it is now possible to date reliably the time of crystallization of igneous rocks and the time of formation of metamorphic rocks. As a result of such measurements made here and by workers elsewhere, it has been found that within geographically large regions all the igneous and metamorphic rocks were formed about the same time. In the Grenville subprovince of Ontario the almost uniform occurrence of rocks approximately 1000 million years old has been known for some time. Similarly, in the Appalachians all the rocks that have been measured give an age of  $300 \pm 100$  million years. Data obtained here and by workers in other institutions show the presence of a large belt of 2500-million-year-old rocks extending from Wyoming through Montana, Minnesota, Manitoba, Ontario, and Quebec. In addition, a large region of 1350-million-year-old igneous and metamorphic rocks has been found in southwestern United States as a result of measurements made in this laboratory.

Similarly, areas of 1000- and 2600-million-year-old rocks have been found in Africa, and a group of 2700-million-year-old rocks has been found in Western Australia.

These measurements support the older idea that earth history is characterized by orogenic episodes during which a large belt of the earth's crust is deformed, uplifted, intruded by igneous magmas, and subjected to regional metamorphism. An investigation is being made in order to see whether there are any regularities in the geographic distribution of successive orogenic belts of this kind. Measurements are also being made to find out whether these orogenic episodes of approximately 200 million years' duration consist of a series of short episodes or whether the formation of igneous metamorphic rocks is essentially continuous.

Soon after the Huancayo Magnetic Observatory was established by the Department, in 1922, the records of geomagnetic variations obtained there showed that the amplitude of the quiet-day diurnal variation,  $S_q$ , in the horizontal magnetic component,  $H$ , is abnormally large. This abnormally large diurnal variation in  $H$  is due to the existence, during midday, of a band of concentrated electric current flowing eastward in the ionosphere over Huancayo. This current, known as the equatorial electrojet, is superposed on the current system responsible for the normal quiet-day diurnal variation,  $S_q$ .

To determine the height, intensity, and the pattern of the current flow into and out of the electrojet it is necessary to determine the variation, with latitude, near the magnetic equator, of the amplitude of the diurnal variation in the three components of the geomagnetic field. As its contribution to the U. S. International Geophysical Year effort, the Department carried out, from March to May 1957, with the co-operation of the Instituto Geofísico de Huancayo, a survey on the west coast of Peru, to obtain data for answering these questions. In addition, locations were

chosen for recording continuously the geomagnetic variations during the IGY. These data will indicate whether the same,  $S_q$ , electrojet is responsible for the large lunar diurnal variations at Huancayo, and whether "electrojet" effects exist for the "sudden commencements" of magnetic storms and for other magnetic variations.

In nuclear physics a study has been made of the angular correlation between the directions of emission of the proton and  $\gamma$  ray in an  $\alpha$ -particle-induced reaction on fluorine. The results show that there is indeed a definite correlation, that somehow the residual neon nucleus remembers the direction in which the proton was emitted, for a sufficiently long time to allow the  $\gamma$  ray to be emitted in a definite direction with respect to the proton direction even though the natural period of nucleon motion within a nucleus is very much shorter than the decay time for the  $\gamma$  ray. The actual form of the correlation shows in several cases a surprising agreement with the predictions of a simple direct or surface interaction mechanism.

In the work of the biophysics section it is increasingly apparent that it is now timely to attempt to interpret the chemical activities of the cells in terms of cellular structures. Kinetic models of the bacterial cell which take into account the known structures of the cell have been considered in detail. One model which fits the present data suggests that there may be a kinetic relationship among the various classes of particles found in bacterial cells, that one type of particle grows and becomes a different type. Experiments to test this hypothesis have been started. The data have not yet had sufficient refinement to test the detailed predictions of the model, but

they do show marked differences in the rates at which radioactive materials appear in different cellular structures. Another feature of one of the models is the assumption that the particles are arranged within the cell in a definite spatial array. Although this feature is difficult to test experimentally it has received some support from an unexpected direction. Clear solutions of bacterial juices were observed to give rise to cell-like bodies containing protein and nucleic acid. Those forms that "reconstitute" from the much smaller particles of the fluid may quite possibly be a different expression of the forces that organize the material of the cells.

That the mechanism which inserts amino acids into the peptide chains of protein operates with a high degree of accuracy is shown by studies of amino acid sequences. Recent work at the Institut Pasteur showed, however, that the mechanism was not perfect, and that certain analogues of amino acids could be incorporated. The resulting proteins were sufficiently altered so that normal growth was not possible. During the year, studies carried out here in collaboration with Dr. G. N. Cohen, of the Institut Pasteur, showed that methionine could be completely replaced by its selenium analogue. In this case the resulting altered proteins still have a sufficient enzymic activity to permit continued growth. These results show that some errors in the formation of proteins are acceptable, and the mechanism of amino acid selection does not have to be perfect. Studies along these lines are being continued to determine whether the degree of substitution is the same in all proteins or whether it varies from one protein to another.



## EXPERIMENTAL GEOPHYSICS

## RADIO ASTRONOMY

*B. F. Burke, W. C. Erickson, J. W. Firor, H. L. Helfer, H. E. Tatel, M. A. Tuve, and H. W. Wells*

## RADIO EMISSION FROM THE SUN

It has been the expectation that our understanding of the solar atmosphere will be increased by a search for relationships between the radio emission from the sun and features of the sun observed optically. Almost always the attempt has involved measurements of one feature of the radio emission and some selected feature of the optical observations: for example, measurements of the total emission from the whole sun at one radio frequency and the sunspot number or the sunspot area or some weighted average of the two. Although the relation between the spots and the radio emission was never found to be close, the matter has been pursued to the point of trying to determine, statistically, from which spot on the disk the radio emission comes or, by extrapolation of statistical data, what would be the level of radiation in the absence of spots. Most of the conclusions reached in this manner have later been found to be incorrect; and, further hindsight shows, many mistakes would have been avoided by having in hand a more complete radio description of the phenomenon before the optical connection was attempted. In the examples mentioned, a measurement of the position on the solar disk of the source of radio radiation would have guarded against the incorrect conclusions.

Although many characteristics of the solar radio emission that could be measured would add to the radio description of the sun, the measurement of the positions on the disk of the sources of the radiation, or in other words the distribution of brightness on the solar disk, seemed to us to be the one that would lead most directly to fruitful optical comparisons.

The problem of designing an antenna for studying the solar brightness distribu-

tion and the behavior of localized bright areas on the sun is different from the problem of preparing to search for new radio stars or to measure accurately the position or intensity of radio stars. When searching for stars the investigator must be able to scan large parts of the sky to see many sources, to detect weak ones, and to give a single position in the sky for each. In the solar case a single strong object is being studied, and arrangements to make measurements on this one object continuously, or at least repeatedly, during the day are necessary. On the other hand, advantage may be taken of the fact that there is only one source like the sun in the sky, and the antenna pattern may have considerable positional ambiguity—the measurements may be consistent with many different positions in the sky as long as only one of them falls near the known position of the sun.

An antenna designed especially for solar studies was used by Christiansen in Sydney for several years to determine the brightness distribution of the quiet sun. His arrangement was an array of 32 small paraboloids in a line 1000 wavelengths long and operated at a wavelength of about 20 cm. All the elements were connected together in phase. The resulting receptivity pattern in the sky was a number of narrow parallel lobes, about 3' of arc wide and spaced about 2°. These fan-shaped beams remained fixed in the sky, and the sun, moving across the sky in its diurnal path, was scanned by each beam (lobe) in turn. As used by the Australian group, this antenna permitted bright areas on the sun to be recognized and allowed for in trying to deduce the distribution of brightness of the quiet sun.

Such an antenna arrangement can clearly be utilized for studying bright areas as well as for eliminating their influence. Each scan of the sun by one of the fan beams gives a position line across the sun for any bright areas as well as the inten-



sity of the region. Later scans then reveal changes in position or intensity.

The selection of the wavelength depends on the end in view. For studying the quiet-sun radiation, almost any wavelength in the radio-astronomy range is of interest, for only when the results from a wide range of wavelengths are available will it be possible to derive a radio picture of the chromosphere and the corona. A similar statement could be made for the active radiation from the sun—the localized bright areas and the bursts. The active radiation is very complex, however, not only in its variation with wavelength, but also in its polarization, variation with time, and change in position, so that it is advantageous to have a number of different types of measurements made on a single active event at similar wavelengths. For this reason a wavelength near to that used by other solar investigators in the same hemisphere is desirable.

An antenna meeting most of these requirements has been built at our River Road site near Seneca, Maryland. The array is modeled after the Australian one, but employs a longer wavelength (90 cm; 328 mc/sec) and different receiving elements. The individual elements are pairs of 10-turn helices mounted on a common ground screen. One helix is mounted with a half-turn rotation with respect to the other and so is out of phase. The two helices can thus be connected to the two sides of a balanced transmission line. (See fig. 1, pl. 2, facing p. 148.)

At present the array has 30 of these elements and is a little over 600 wavelengths long. The measured beamwidth of one of the fan beams is 4.8' of arc to half-power points, and the fans are spaced  $2\frac{1}{2}^\circ$ . A comparison-type receiver compares the power received by the antenna with that from a room-temperature resistor at a 1000-cycle rate.

Four scans of the sun taken near noon on successive days are shown in figure 2. Two bright regions can be seen moving across the disk as the sun rotates. One of

them is variable in time—changes take place during the few seconds required for the beam to scan it. The other region is not only steady during the scan but also is much the same day after day. For the steady regions the apparent motion of the source across the solar disk can be derived from a series of these scans, and a height above the photosphere can be found for the source. For the more changeable regions, connections will be sought with the

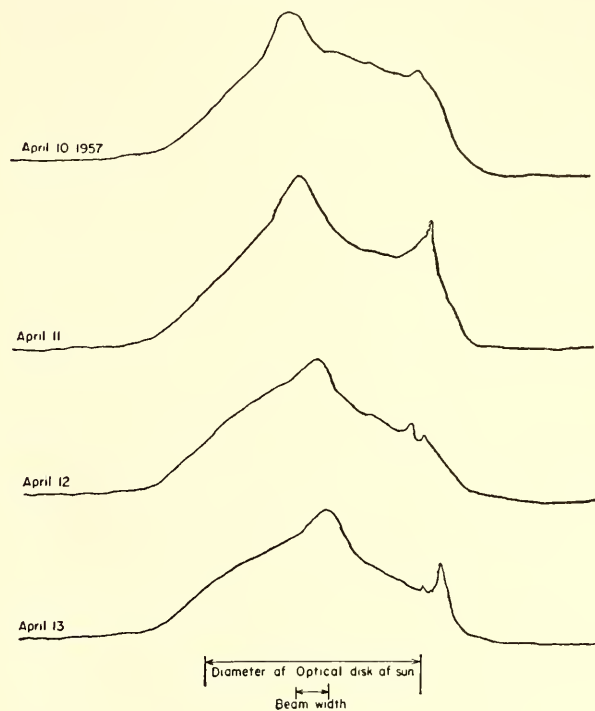


Fig. 2. Scans of the sun on four successive days taken with the helix array. Two regions of enhanced radiation are seen which move from left to right as the sun rotates. One of the regions (near the center) is relatively stable; the other (near the right, or west, limb) changes in intensity in the few seconds required by the narrow antenna beam to scan it.

optically observed features near the correct position line given by the scan. (*J. W. F., W. C. E., B. F. B.*)

#### ABSOLUTE INTENSITY MEASUREMENTS OF DISCRETE RADIO SOURCES

The report for 1955–1956 described preliminary results of intensity or flux measurements on radio stars at frequencies below 30 mc. Greatly increased solar activity of the past year has resulted in an ionosphere which usually is not sufficiently

transparent for additional radio star measurements at the lower frequencies. In all probability, it will be impossible to repeat our observations in the 12 to 15 mc range until perhaps 1963–1965.

A review of intensity measurements of discrete stars in the available radio spectrum has emphasized the very unsatisfactory and inadequate nature of measurements in this important area of radio astronomy. As yet, there is not one accepted standard of reference at any frequency. Even in measuring the same star in the same frequency range there is very little agreement among different investigators. Few have reported any flux measurements at more than some isolated frequencies, and those may lack consistent observational procedures. No one has assumed the task of making a careful, systematic series of “absolute” measurements over a wide range of frequencies.

Accordingly, one of our objectives in radio astronomy has evolved into a program of flux measurements on a few of the intense radio stars over a wide band of the useful spectrum. For the purpose of minimizing all errors, both relative and absolute, we are undertaking a series of measurements by systematic and consistent procedures over the frequency range. In theory a flux measurement is simple. The intensity,  $S$ , is obtained from

$$S = kT/A$$

where  $S$  is in watts per square meter per cycle per second,  $T$  is the equivalent temperature of the signal at the antenna,  $k$  is Boltzmann’s constant, and  $A$  is the effective area of the antenna in square meters. In practice, however, there are several potential sources of error.  $T$  depends on (1) precise knowledge of all transmission line losses between antenna and receiver, (2) accuracy of the calibrating system, and (3) the precision of the record scalings. The effective antenna area,  $A$ , is calculated from knowledge of its polar diagram, the efficiency of the ground screen being taken into account. Experi-

ence indicates that the most likely sources of error may lie in the calibration method and in the determination of antenna aperture. Such errors are now minimized by calibrating noise sources against heated resistors at each frequency, applying calibration signals while antennas are also connected to the receiver, and using the simplest of antennas (dipoles) for all primary observations.

The use of dipole antennas limits the primary observations that can be made at this latitude to one or two relatively isolated radio sources. Even with one primary measurement at any frequency, however, higher gain arrays with adequate resolution are then employed to obtain relative intensity measurements from which an accurate flux may be determined for other radio stars.

At frequencies below approximately 100 mc we have made dipole (primary) measurements of both Cassiopeia A and Virgo A. We plan to continue dipole measurements to the limit of sensitivity, which may be several hundred megacycles. For observations at higher frequencies it will be necessary to standardize other antennas of larger aperture—perhaps horns—against dipoles at the transition frequency and to use scaled versions of the large antenna in subsequent operations. In this manner, no difficulty is anticipated in extending the series of absolute measurements to 1000 mc or more.

The present series of measurements includes 18.5, 27, 50, 87, and 108 mc. The sources of principal interest are Cassiopeia A, Cygnus A, Taurus A, and Virgo A. Some of the final primary observations on Cassiopeia at 87 and 108 mc are scheduled for July 1957, at which time the source will be transiting just before sunrise. The next recording frequency is 207 mc. Advance preparations are nearing completion, and observations are scheduled to start in late June or early July 1957. The next step will be to about 300 mc or higher, depending on the 207-mc results.

When our measurements are combined



with all other known values, several interesting characteristics promptly become apparent: (1) there appears to be a "knee" or change in slope of Cassiopeia A below 50 mc; (2) there is so much scatter of points in the range 200 to 600 mc that earlier curves showing a "flat" spectrum may be seriously questioned; (3) at frequencies up to approximately 1000 mc the ratio of Cassiopeia A to Cygnus A is decreasing (Cygnus A is getting relatively stronger at the higher frequencies), but the ratio of Virgo A to Cygnus A is essentially unchanging over the same range. In connection with the ratio measurements it is perhaps significant to note that the ratio of two extragalactic sources (Virgo A and Cygnus A) is steady but the ratio of Cassiopeia A, which is a galactic source, to Cygnus A is changing in a manner to indicate that the very distant source in Cygnus A is getting relatively weaker at the lower frequencies. Absorption in interstellar space—a rough distance scale—is one of several possible explanations of the trend. (*H. W. W.*)

#### PRECISE POSITION APPARATUS

The optical radiation from an astronomical radio source is capable of yielding a great wealth of information that the radio radiation cannot give. Therefore, to obtain detailed knowledge of the nature of radio sources, optical identification must be made. Up to this time, however, a discouragingly small number of radio sources have been unequivocally identified with optically observed objects. These sources represent a great variety of objects, ranging from the sun and planets, through supernova remnants, and peculiar galactic emission nebulosities, to external galaxies. Only a few objects of each class have been identified. Before definitive conclusions about the general nature of radio sources can be drawn, many sources of each class must be identified. It is impossible to carry out any extensive astrophysical studies using radio information unless the state of aggregation and other physical properties

of the gas or matter emitting the radio waves are known. Some sources have steep density gradients to high pressures, as in the solar atmosphere; others appear to be extremely tenuous and extended gas clouds; some are relatively much cooler than others; all appear to be highly turbulent. Until much larger numbers of the various types are known and studied by optical methods we can hardly consider radio observations to have contributed much to astronomical knowledge. Moreover, faint but easily observable radio sources may lie beyond the radius of the optically observable universe. Therefore, the statistics of faint radio sources may yield information about the large-scale structure of the universe.

In order to extend the list of optical identifications, precision position determinations for a large number of radio sources must be made. The few identifications so far have proved to be interesting objects, but if meaningful identification with objects as faint as 18th magnitude are to be made, radio positions should be measured to the order of several square minutes of arc, a precision attained so far only for the most intense sources. The strongly felt need for a greater number of precise positions has led us, therefore, to investigate the problem. An antenna constructed for precise position measurements must possess not only the necessary mechanical and electrical stability but also sufficient angular resolution to guarantee that most of the sources measured are truly discrete, not simply blends of several sources (often referred to as "confusion").

A long array, to give a narrow "line of position" in the sky, or an interferometer composed of a pair of long arrays along one line, appears promising, particularly in view of the success of the 328 mc/sec helix array we have in use on the sun. The arrays must be oriented in several different azimuths, since a single orientation gives high precision for only one coordinate. At least three different orientations of the arrays appear desirable, in



order to overdetermine the position and guard against confusion errors.

Several small arrays and a receiver were constructed for use at 400 mc/sec to aid in design of the large arrays with the aim of achieving position accuracy within 4 square minutes of arc. These arrays are expected to be made in small sections, each about 10 feet long to facilitate remounting along different lines of azimuth. The most suitable element investigated so far is a  $60^\circ$  V reflector excited by a line of full-wave dipoles. The test arrays used open-wire feeder lines which can be constructed to give low losses and good phase stability. Trial interferometric measurements of right ascension were made with small arrays and a short baseline (50 meters) on the strong sources in Cygnus and Cassiopeia, the results agreeing with the known positions to within a minute of arc. Preparations have been completed, and the construction of a large array is in progress. (*B. F. B., J. W. F., W. C. E.*)

#### RADIO EMISSION FROM JUPITER

Regular observations of the planet Jupiter were not continued, but data taken in previous years at this laboratory were re-examined in the course of preparing a summary of the present status of this phase of radio astronomy. In the previous report it was mentioned that the region of the planet which appeared to be the most persistent source of noise during 1955–1956 was certainly not the same as that reported by Australian workers from their pre-discovery records taken from August to September 1951. An analysis of the time dependence of all obtainable observations, including the Australian observations in late 1950 and early 1951, the August–September 1951 Australian series, the pre-discovery observations at this laboratory during June 1954, the Mills Cross series from January to May 1955, and the 1955–1956 Carnegie observations, revealed that the most active region could always be reconciled with a single center of activity

on the planet, having an approximately uniform rotational period of  $9^h 55^m 28.5^s$ . Interestingly, none of the visual observations report any surface features exhibiting this rotational period; the source of radio radiation therefore probably lies below the cloud level, and might well be associated with the surface of the planet itself. (*B. F. B.*)

#### SEARCH FOR VENUS

During the fall of 1956, when Venus was at elongation, two interferometers were placed in operation to check on the validity of the reported low-frequency non-thermal radiation from this planet. These interferometers operated at frequencies of 22 and 26.75 mc/sec. The 22 mc/sec array consisted of two elements, each containing eight half-wave dipoles, which were phased in such a direction that Venus rose through the beam about 2 hours before sunrise each morning. Each element of the 26.75 mc/sec array consisted of four half-wave dipoles phased in a similar manner. The observations were attempted before sunrise, since after sunrise strong interfering signals of terrestrial origin make identification far more difficult. Observations were made continuously from September 19 to October 25, 1956, but no radiation of Venusian origin was found. If during this period Venus had been a source of equal intensity to the Crab Nebula, it would have easily been observed.

During these observations, one interesting effect was noted. Approximately 30 minutes before sunrise, just as the interfering signal strengths were rising, a few lobes of an interference pattern were often found at both frequencies. They could not be associated with radiation originating at Venus, since they disagreed both in phase and in period with radiation coming from the direction of that planet. The effect may be due to the build-up and movement of ionized regions in the atmosphere as they are illuminated by sunlight, some of these regions being responsible for the reflection of the interfering radiation, which

may be of terrestrial or solar origin, to the observing site. (*B. F. B., W. C. E.*)

Other interferometer records of the summer of 1956 using simple dipoles at 18.5 and 26.75 mc were scanned in the search for Venus. No events at either frequency could be uniquely identified as having origin in Venus, although several interesting borderline occurrences were noted on the 18.5-mc instrument. These tests indicate that no pronounced signals from Venus were received, but do not conclusively prove the absence of some weak emissions at random intervals. (*H. W. W.*)

#### RADIO HYDROGEN

During the past year a large part of our effort in the 21-cm hydrogen-line program has been devoted to the improvement of our observing equipment. We have designed an equatorial telescope mount suitable for large dishes, installed a multi-channel spectrometer, and improved the over-all stability of our recording equipment. In addition to this emphasis on instrument improvement, however, we have continued our measurements at a modest rate. Several more meridian galactic surveys have been finished, and a thorough survey of the Pleiades and the II Persei clusters is nearing completion. One finding is that a large region of the sky in the direction of these clusters is covered by a homogeneous hydrogen gas cloud. Preliminary examination of the Doppler-shift cross section of this cloud indicates that a Gaussian velocity distribution represents the major portion of the observed intensity. The velocity distribution parameter is 5 km/sec, corresponding to 600° K, whereas the "spin temperature" is assumed to be about 150° K. It should be noted that the optical absorption of interstellar lines indicates that this mass of gas is probably 150 to 350 parsecs distant.

The general problem of the design of large parabolic antennas was resolved into two principles: astronomical uses make it desirable to have an equatorial mount; and the structure should be designed with

rigidity commensurate with the precision of the reflector. Hence the main drive gears and major structural units should be large, so that machining tolerances can also be large. Numerical studies of our resulting initial design showed it to be adequate and economical for supporting reflectors as large as 85 feet. The National Science Foundation is planning to build a large "national facility for radio astronomy" at Greenbank, West Virginia. Hence we carried out some careful studies of the features required for larger parabolic reflectors and mounts. A 200-foot bar of steel first compressed and then stretched by its own weight will elongate about 0.4 cm. Since any major structural unit must support more than its own weight, its elongation will be greater. If it supports  $2\frac{1}{2}$  times its own weight the elongation is 1 cm. In a reflector the depth is much less than the breadth, so that major structures have even larger loads than simple trusses of equal width and breadth—perhaps twice as much. If the deflection becomes 5 cm it reaches (exceeds) the limit for a reflector intended for 21-cm waves. This very rough calculation, which neglects some features such as "hoop strength," indicates that a simple cantilever structure of steel cannot be expected to be useful as a steerable astronomical instrument if it is much longer than about 200 feet. For larger structures other features of design must be devised to surmount the problem of the elasticity of steel. The economic problem is also considerable. Devices of this magnitude constructed with present techniques are expected to have costs in the range of perhaps 10 million dollars.

Our hydrogen-line radiometer has been converted to a multichannel device. It is now used to record in 54 channels 10 km/sec wide, spaced at frequencies equivalent to a Doppler shift of 4 km/sec between channels. The basic system is a Dicke-Ewen comparison receiver with two local oscillators alternately switched on and off. The oscillators are tuned separately, so that the two receiving frequencies and their



differences can be adjusted with precision. The received signal, detected in a crystal, is doubly converted to a band extending from 1.5 to 2.5 mc/sec, at an output level of 2 to 3 volts rms. A common output amplifier drives 54 separately tuned filters. At the output of each filter is an individual amplifier with ample feedback stabilization.

The signal detected at the output of each of these radio-frequency amplifiers contains the switching frequency (about 450 cps). It is amplified in a feedback-stabilized audio amplifier and detected in a phase-sensitive (450 cps) detector. The output of the final amplifier on each channel is 2 volts rms. Between this output and the final detector is a potentiometer gain control for each individual channel. The phase detectors charge 1- $\mu$ f condensers through 400 or 4000 megohms, depending on the desired output time constant. The voltage on the storage condensers is read out by means of an electrometer tube and recording potentiometer. Read-out time is 2 minutes for the 54 channels. This system has very great advantages over the single channel unit with frequency scan, but there are several features that we wish to modify. The output diodes have a stability of about  $\frac{1}{2}$  millivolt per day, which is equivalent to a signal of  $1^\circ$  K at the antenna, so that the diodes need adjustment every two or three days. We therefore expect later to increase the audio gain by about a factor of 8. The gains of the feedback amplifiers are difficult to monitor; we calibrate the over-all system with a modulated noise diode at the input, and statistical fluctuations during the calibrations are important. Our wide-band amplifier (1.5 to 2.5 mc/sec) does not have a sufficiently low impedance to drive the high capacity of the existing cables feeding the 54 filters, so that we are forced to operate at a low output diode level. These cables must be replaced by a low-capacity feed.

The whole system is flexible enough to use in many problems. The oscillators can

be tuned over a wide range. Channels can be connected in parallel so as to have greater bandwidth and smaller fluctuations. We are now in the process of developing the observing procedures for the best use of this powerfully analytical instrument.

Another problem of great difficulty and even greater importance is the zero stability of the detection system. Many interesting problems require the detection of minute signals of the order of  $1^\circ$  K antenna temperature, or less. Such measurements require a system with zero drifts and changes not exceeding  $0.5^\circ$  or  $0.3^\circ$  K over periods of several hours. Our detection system last year had a variable stability with occasional fluctuations even as high as  $10^\circ$  K over a run of several hours. After much testing and many changes, including the physical separation of the local oscillators from the receiver units and improvement in the shielding of transmission cables, we have been able to reduce the zero changes to a drift of less than  $1^\circ$  K per week on the dummy antenna. On the sky antenna the system is not as free of drifts; at times it may shift as much as  $\pm 1^\circ$  K in several hours. For the narrow-band short time constant (7 minutes) this amount of drift is not important, but in looking for signals of a few tenths of a degree Kelvin it is still a limiting factor.

Using this new multichannel recording instrument we have in progress a repetition and extension of our survey of the hydrogen clouds within  $\pm 20^\circ$  of the galactic plane, for a great many points spaced from  $2^\circ$  to  $10^\circ$  apart along the galactic equator as visible in Washington. Residual hydrogen is also found at all points examined to date at higher galactic latitudes. (*H. L. H., H. E. T., M. A. T.*)

*Tidal distortion of the galaxy.* The Leiden and Australian groups have shown that, if the Lund galactic pole is shifted, the major concentration of galactic hydrogen lies close to the plane, with small deviations. The deviation for the outermost spiral structure is noticeably greater,



and the 21-cm meridional surveys taken at this laboratory were examined to see whether the effect was systematic. Surveys were available here, at galactic longitudes  $50^\circ$ ,  $60^\circ$ ,  $80^\circ$ ,  $90^\circ$ ,  $110^\circ$ ,  $180^\circ$ ,  $200^\circ$ , and  $210^\circ$ , which showed that in general the center of mass of the outermost hydrogen lay consistently below the plane on the side closest to the Large Magellanic Cloud (LMC), but above the plane on the more distant side, except for longitude  $110^\circ$ . Although the effect is qualitatively in the proper direction to be due to tidal effects of the LMC, the observed deviation appears to be much too large if conventional values for the masses and distance scales of the galaxy and LMC are assumed. At  $l=210^\circ$ , the deviation is about 300 parsecs, which is approximately 20 times larger than would be expected from gravitational effects, if Schmidt's galactic model is used, together with a mass of  $4 \times 10^9$  solar masses for the LMC. The effect is sensitive to the assumed force perpendicular to the galactic plane, but it appears that both a larger mass for the LMC and a larger distance scale for our galaxy are required if the effect is to be explained using gravitational forces only. (B. F. B.)

### THE UPPER ATMOSPHERE

*H. W. Wells*

#### WINDS AND RADIO STAR SCINTILLATIONS

A paper, "Large scale movements of the layers," was presented to the AGARD (Advisory Group for Aeronautical Research and Development) at Oslo, Norway, in July 1956. The activities of this Department and others were reviewed in the light of additional analyses. Sweep-frequency observations of the ionosphere at networks of stations spaced 20 to 200 miles reveal the nature of apparent large-scale movements and permit a three-dimensional interpretation. Analyses clearly show for the first time that traveling disturbances have vertical as well as horizontal components of motion. The disturbance wave front is often inclined about

$45^\circ$ . Such large-scale drifts are predominantly downward and into the east. Often there is no significant change either in velocity or in direction as the disturbance progresses downward through the outer atmosphere. Effects such as described could be caused by an inclined wave front moving horizontally, an inclined wave front moving vertically, or combinations of both motions. Predominant velocities are between 100 and 200 m/sec.

In another review of ionospheric winds from other radio methods of measurement, an intercomparison was made of results from the "meteor-Doppler" and the "fading" techniques. The outcome was the interesting fact that the two methods independently establish similar characteristics of *E*-layer winds. The outstanding features are (1) velocities in the range 50 to 100 m/sec, (2) large semidiurnal components with clockwise rotation of wind directions in the northern hemisphere, and (3) counterclockwise rotation in the southern hemisphere.

Radio star scintillations have been observed at all operating frequencies between 18.5 and 108 mc. Although the scintillations at 108 mc, which is the IGY satellite frequency, are not a normal occurrence, it is clear that varying refraction in the ionosphere causes the apparent "radio" position of an object to change by significant amounts. At 50 mc, scintillations were prevalent during the period of observation. At 27 mc, preparations have been made for the operation of a three-station network to measure the drift characteristics of the ionized clouds producing the scintillations.

To explore the possibility of long-distance propagation of electromagnetic waves from power lines some brief experiments were conducted at the Derwood Field Station. It was assumed that any peak in radiation at 50 cycles would indicate foreign origin. Filters were incorporated so that the strong 60-cycle radiation from domestic sources would not interfere. The recordings showed the presence of

very substantial energy between 45 and 55 cycles without any peak in the 50-cycle region. Random atmospheric noise and other disturbances left a high residual noise level, however. A few isolated cases of activity during thunderstorms clearly established the fact that electromagnetic radiation of these extremely long wavelengths is generated at such times.

Preparations for the XIIth General Assembly of the International Scientific Radio Union at Boulder in August–September 1957, and attention to many features of the program of the International Geophysical Year, especially in the areas of ionospheric physics, have occupied some members of the DTM staff during a considerable part of the current report year.

#### THE EARTH'S CRUST

*L. T. Aldrich, J. W. Graham, H. E. Tatel,  
M. A. Tuve, and G. W. Wetherill*

The exploration of the earth's crust continues to present us with problems of great interest. Our general goal, of course, is a clear recognition and understanding of the many large-scale physical processes that, operating during the long periods of geological time, have resulted in the present conspicuous features of the earth, such as the continents and the ocean depths, mountain ranges and high plateaus, and the distribution of land and ocean areas. We are also interested in other matters like the equatorial bulge, the stability with time of the position of the geographical poles, and the time scale of geological uplift and erosion. Perhaps we may some day have better notions about the remote and the immediate "causes" of such processes as mountain building and the cyclical immersion of large continental areas under shallow seas, but clearly the first necessity is a comprehensive and quantitative description of the earth as it is today. In particular, we need data on relatively inaccessible matters, like the horizontal and vertical density distribution of the rocks under the continents and under the oceans. We have directed our efforts toward such questions

in the seismic program with explosion waves. Our work on isotopes in rocks aims at establishing bench marks for a time scale in Precambrian geology.

#### SEISMIC STUDIES

The mathematical picture, for gravity and seismic computations, of an earth's crust comprised of a series of horizontal rock layers, each several kilometers thick and of successively increasing density, has given way in very recent years to a much less specific picture, namely, a crust with horizontal and vertical inhomogeneities, and with average characteristics that do not change by large amounts over short horizontal distances.

In Alaska we find a crust that has a mean seismic velocity uniform with depth to the mantle. It is easy to imagine, then, as appears to be increasingly acceptable among students of earth structure, that the change in velocity is the result of a "phase transition," a change of chemical crystal lattice relations and resulting change in density without change of chemical composition. But if this is actually true, why is the transition only 11 km under the ocean surface and 30 km under the continental surface? The puzzle is even more perplexing in view of the recent heat-flow measurements through the ocean bottom in the Atlantic and the Pacific. The oceanographers find, contrary to expectation, that ocean and continental heat flows upward through the rocks of the crust are almost the same. This being so, it can hardly be an equilibrium temperature and pressure distribution that determines the depth of the velocity discontinuity, and hence it seems improbable that this abrupt change from crustal to "outer mantle" rocks can be a phase transition, *unless* the present heat flow is in part an indicator of changing heat flow and changing temperature distribution.

#### *Velocity versus Depth*

We have tried to fit the crustal data obtained from our measurements on the seis-



mic waves from explosions during the past decade to various types of possible crustal structures, treating the problem as one in geometric optics in which the velocity varies in different specific ways with depth. Our conclusion has been that the compressional velocity does increase with depth in some manner, probably at an increasing rate, from a mean surface value of about 6 km/sec to a value of about 7 km/sec at a depth of, say, 30 km, and, just below this, abruptly to about 8 km/sec, the compressional velocity of sound waves in the outer mantle. These conclusions were based upon the geometrical optics approximation for sound waves, which has been the only complete theory available. We have always noticed, however, that certain expected arrivals have not been observed, for example the refracted wave expected between 100 and 180 km. This failure has been excused on the hypothesis that the "reverberation" level, due to interconversion of wave types at topographic and subterranean boundaries, is unfortunately enough to obscure these arrivals. This may still be so.

The Alaskan measurements (Year Book 54, 1954-1955) are one of our more precise sets of data. They show well developed total reflection from 80 to 200 km and strong refracted waves from 160 km out. These values are well fitted by a simple crust of a single layer of rock in which the velocity is constant with depth down to 31 km, at which point it jumps abruptly from 6.1 to 8.1 km/sec. With this simple structure the critical reflection should have been observed at 70 to 72 km; in actual fact it was observed strongly at 88 km and weakly at 80 km from the shot.

A model experiment carried out in the laboratory using impulses of microsecond duration on a two-layered medium of brass and iron showed the same effect; namely, no refracted wave was visible above the reverberation level at distances beyond the critical reflection where it would be expected (though perhaps weak).

The distance from the impulse point to

the critical reflection, calculated on the basis of Snell's law and the measured velocities, is appreciably (10 or 15 per cent) less than the observed distance to the abrupt appearance of the critical reflection of the compressional wave. This is just what we observe in field work, but in the laboratory experiment we know that the "crustal" velocity did not increase with depth.

There is at present no theory describing the nature of a critical reflection of a compressional pulse in such a medium as the earth. As a rough approximation, the critical reflection pattern could be equated to that obtained by the masking of a point source by a large disk, the apparent source being the mirror image of the true source at the surface. The solution to even this problem does not exist for light waves. Since the source and image are not a great many wavelengths apart in our seismic work and also in the laboratory model, it might be imagined that there would be a kind of Fresnel interference at the edge of the excluded disk (due to compressional waves entering the mantle within the circle defined by Snell's law). Such an interference pattern, strongly dependent on frequency, would predict an intensity that only begins to rise at the geometrical shadow edge (that is, at the critical reflection distance defined by Snell's law), and increases to a maximum at a distance well beyond this. The lateral extent of this interference zone can be estimated as follows: The minimum length of the observed critical reflection pulse in seismic field work is 0.15 to 0.25 second, and the pulse consists of three undulations, up-down-up. If there is interference, it must occur between at least two Fresnel half-wave zones generating, say, two of these undulations 0.12 second apart. For these, the distance  $R$  from source (mirror image) to surface must differ by  $\frac{1}{2}\lambda$  ( $\lambda$ =wavelength). That there is complete interference at a point means a lesser degree of interference along the surface for a distance of, say,  $\delta$ . By simple geometry, the dis-



tance, perpendicular to a (slanting) ray path, is  $\sqrt{R\lambda}$ , and along the earth's surface  $\delta$  is about  $1.5\sqrt{R\lambda}$ .

Typical values obtained in Alaska give  $R \sim 90$  km,  $\lambda = 0.12 \times 6$  (km/sec) = 0.7 km, leading to a value of  $\delta$  of about 12 km. Thus the spread of the interference pattern along the earth's surface would be expected to be in the neighborhood of 12 km. In this interval the amplitude of the "critical reflection" would be expected to increase with distance from the source from a low to a high value. The reflected pulse is therefore expected to be weak at the critical distance calculated from the ratio of velocities by Snell's law and could be obscured by the reverberation background. Thus the total reflection might not be observable until well beyond the critical distance predicted on a simpler basis without the interference effect. Though the estimate is crude, it is based upon well known principles and probably gives the correct order of magnitude of the interference effect. The difference between 70 km as calculated for the critical reflection distance by Snell's law from the observed upper and lower velocities and the observed distance of 88 km when the strong reflections first appeared is close to the added distance estimated by this crude approximation.

Hence, the system of reflection observed on our Alaskan expedition is indeed that which must be expected from the simplest form of a single layer of crustal rock overlying the mantle, a crustal layer having a constant velocity, that is, no change of velocity with depth. The conclusions previously reached about the velocity distributions in other regions will have to be re-examined, as we have previously accepted the first appearance of the critical reflection at distances beyond where it was expected, on the basis of Snell's law, as evidence of moderate increase of velocity with depth. In general, the increases in velocity with depth that we have previously deduced will be diminished. They have not been large, and this new interference prediction may wipe them out, leav-

ing velocity constant with depth in the crust, at least in most places. (*H. E. T., M. A. T.*)

### *Gravity and the Earth's Crust*

The literature contains about two thousand measurements of gravity whose isostatic corrections have been calculated. These serve as the basis of the general belief in Airy's hypothesis that high mountains are supported in hydrodynamic equilibrium by a thicker crust floating in the outer mantle of heavier rocks. Our seismic measurements on the Colorado Plateau and in Alaska (Year Book 52, 1952-1953; Year Book 54, 1954-1955) have shown that high land areas and thick crustal regions do not necessarily go together. The question then arises whether or not there is disagreement between the two sets of measurements, gravity and seismic.

An analysis of the gravity data shows that the data are much too few to give detailed crustal information. If all the uncertainties are taken into account, and all the data averaged together to obtain a mean world-wide crustal depth with minimum apparent statistical error, the depth turns out to be  $30 \pm 20$  km. This can hardly be considered an accurate specification of the depth to the mantle. For any smaller portion of the earth's surface, the statistical situation is even worse. The reasons for this inaccuracy in the specification of mantle depth from gravity measurements do not seem to be widely recognized. There are two: the data are few; gravity values are not sensitive to the "depth of compensation" (the depth at which the excess weight of the mountains is exactly balanced by the defect of weight due to flotation in the heavier rocks below).

The gravity measurements show wide-scale isostatic and free air residuals of the same size. Some of these regions have dimensions of a few hundred kilometers. The origin of the residuals is not specifically known. They indicate wide-scale crustal inhomogeneities, or perhaps a horizontal distribution of inhomogeneities in

density in the upper mantle rocks. Further measurements of gravity may give us some better basis for judgment about the nature or cause of the residuals, and possibly some indications concerning outer earth structures. (H. E. T.)

### *International Geophysical Year*

In co-operation with the U. S. National Committee for the International Geophysical Year we are organizing a seismic expedition to South America. Using the waves from the large explosions regularly set off in the copper mines there, we will endeavor to explore the crustal structure and measure the crustal depths under the high Andes and the coastal plains. The mean heights are about 10,000 feet near the large copper mines of southern Peru and northern Chile. We therefore hope to be able to make crustal measurements under regions of great topographic heights, permitting a more definitive study of the effect of topographic height on crustal depth than we were able to achieve in our efforts on the Colorado Plateau and in Alaska.

Much effort has been expended on the complex arrangements necessary for an expedition of this nature. Outfitting six vehicles for three months of measurements, many of them in locations without roads, is an arduous and time-consuming task.

*Instrumental development.* The seismic instruments we have designed and made have proved rugged and dependable. They are in general sufficiently sensitive and fairly portable. In some places, however, the ground unrest is small enough so that amplifier input provides the limiting sensitivity. In others there are special opportunities to observe which we miss because of the bulk of the equipment we must carry. We have therefore constructed new seismometers and prototype transistor amplifiers for increased sensitivity and decreased bulk.

The new seismometer has a pendulum mass 100 times the older seismometer (the total weight of this new unit is 14 pounds).

With this instrument the input noise voltage of the amplifiers (usually 1 to 2 microvolts) will be one-fifth to one-tenth the voltage produced by ground unrest in the quietest spots of quiet regions.

The new transistor amplifiers (developed by E. T. Ecklund, of our staff) are small, compact, and rugged. The circuits are stabilized by degeneration so that the power gain (1,000,000) is largely independent of temperature and battery voltages. The input fluctuation voltage is comparable with but not quite so good as that of a vacuum-tube system. The combination of the new seismometer and transistor amplifier, however, is far more advantageous in signal noise and weight than the system we have been using to date. (H. E. T., M. A. T.)

### ROCK MAGNETISM

*J. W. Graham*

Within the past few years the challenges presented by the subject of rock magnetism have been taken up vigorously by approximately a dozen research groups in many countries of the world. It is not yet certain what rewards will accrue from the efforts. Briefly, the subject has become subdivided into two schools: one maintaining that the rock-magnetism data already at hand provide justification for believing that it is now possible to talk with considerable assurance about large-scale geophysical phenomena like continental drift and polar wandering, while the other group holds that the subject is intrinsically so complex that no such important conclusions can be justified in our present state of very limited knowledge. Each school has been able to make a strong, but not convincing, case for its stand.

The efforts at the Department during the past year have centered on the question of the possible influence of stress on the directions of magnetization of rocks. It is at once clear that, in rocks which have been stressed beyond the limit from which they can recover mechanically on being relieved, some changes of magnetic prop-



erties are to be expected. Such rocks, in general, are thought to have been adequately excluded from rock-magnetism studies aimed at questions like continental drift and polar wandering. But a more subtle problem demands serious attention: if a rock is in a state of nonhydrostatic stress at the time it is magnetized (a condition that most certainly has frequently occurred—for example, in sediments magnetized after deposition, and in intrusive igneous rocks), and then the stress is relieved, the magnetic moment of the sample may change to another value, at the time of unloading, by virtue of the property known as magnetostriction. If the magnetization of rocks in general should prove to be particularly stress-sensitive, many of the data supposedly bearing on the problems of continental drift and polar wandering would become immediately suspect, simply on the basis that our knowledge of the physical conditions prevailing during the time the rocks were magnetized, and subsequently, is woefully inadequate.

The basic question of the stress sensitivity, at room temperature and atmospheric pressure, of the magnetization of rocks is being investigated experimentally with new equipment constructed for the purpose. The direction and intensity of magnetization are measured with an astatic magnetometer while the sample is held under compressive stress of about 2500 psi by a simple nonmagnetic mechanical “nut-cracker.” This stress is about the same as would be acting at the base of a column of rock 1800 feet high. Although the ultimate stability and sensitivity of this particular magnetometer have not been achieved (thus precluding studies of weakly magnetized sediments, for example), and the force that can be applied is well below the limit where fracture and flow would begin, nevertheless the observations to date are of sufficient importance and interest to warrant further elaboration. They are being made in collaboration with J. R. Balsley, of the U. S. Geological Survey, and Professor A. F. Buddington, of Princeton

University, utilizing the extensive suite of rocks on which detailed mineralogical, chemical, and magnetic analyses have already been performed.

In this laboratory reconnaissance it has been found that the greatest stress sensitivity is displayed by rocks that contain magnetite as the principal magnetic constituent. If the original natural magnetization of the sample is expressed as an intensity-direction vector, and then the magnetic vector of the stressed sample is observed, a vector resulting from the stress can be inferred. Most, but not all, samples return reversibly to their original condition when the stress is released. For nearly pure magnetite (96 per cent), the length of the stress-induced vector may be as much as 40 per cent of the original vector for a stress of only 2500 psi. In contrast, rocks whose ferromagnetic minerals have compositions between the extremes of ilmenite and hematite are quite insensitive, e.g., intensity change of 1 per cent or less for a compressive stress of 2500 psi. (See figs. 3 and 4.) No general rules covering all rocks can be formulated for the orientation or sense of the added vector relative to the stress direction or the original direction of magnetization. The inference is that the various magnetic components present in rocks are of both positive and negative magnetostrictive signs, and that there can be interaction of the magnetostrictive moments with the natural moment of the sample.

It is worth while to make an estimate of some of the ways in which these findings can be related to what is known of the magnetization mechanism of rocks. The various ferromagnetic minerals that have been recognized in rocks are known to cover a considerable spread in the numerical values that can be assigned to such measurable properties as Curie point, saturation magnetization, susceptibility, and variation of various properties with temperature. It is rare that in any given rock a single magnetic species occurs; usually two or more coexist, and they may



be intimately intermingled or remotely separated as discrete units. Depending on the state of aggregation, there may be con-

phases which slowly during geologic time break down into a mosaic of two or more mineral species. Some ferromagnetic minerals are prone to marked alteration by the simple process of oxidation. It is known that all these features are important in rock magnetism.

To this already complicated picture we now add the question of the effect of stress. Reconnaissance measurements indicate that stresses, which geologically speaking are almost trivial, can have a marked influence on magnetization. The response of the magnetizations to stress is such as to indicate that different species present are making different types of contributions to the change of the magnetization. It probably would be possible to describe the relative magnetostrictive contributions of the different ferromagnetic species in a given sample, but such knowledge gained today would not necessarily have any meaning to the more interesting problem that we have had in view, namely, attempting to infer the past directions, not to mention intensity, of the earth's magnetic field. This is so for two simple reasons: that we can give an accurate account neither of the stress history of the rock nor of the chemical and physical evolution of the magnetic species. It is of course clear that, the older the rock, the greater will be the uncertainty. The only obvious way out of these difficulties is to have so many field observations from so many rocks of so many types in so many settings that the insidious influence of magnetostriction, taken either alone or in conjunction with time-dependent parameters, is eliminated. The prospect is hardly encouraging.

The ultimate geophysical implications of these observations are not yet known, but they certainly do not foster the hope that has prevailed for decades that by way of the techniques of rock magnetism it will be possible to deal effectively with such major geophysical questions as continental drift and polar wandering. The present observations do not assure that such hopes are beyond reach; they do call for great

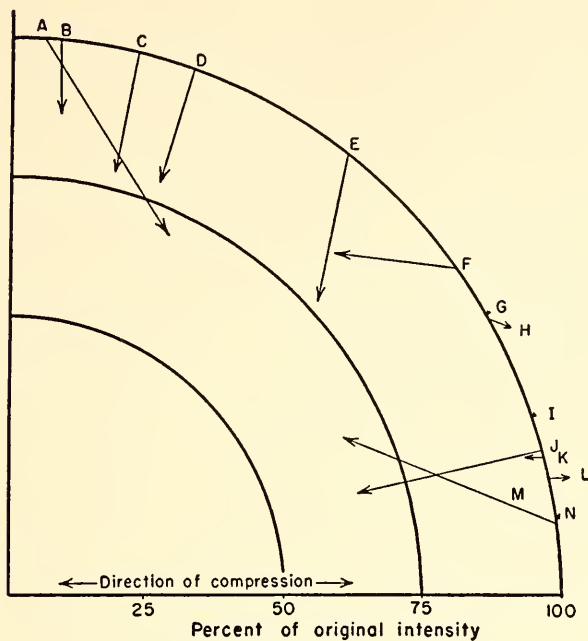


Fig. 3. Figure 3 summarizes the magnetic changes that were produced by increasing the axial compressive stress from 350 to 2650 psi. The results are plotted as vectors: the original intensity at 350 psi is taken as 100 per cent, and its angle relative to the direction of compression is plotted. The arrow end of the vector indicates the intensity and direction of magnetization of the stressed sample.

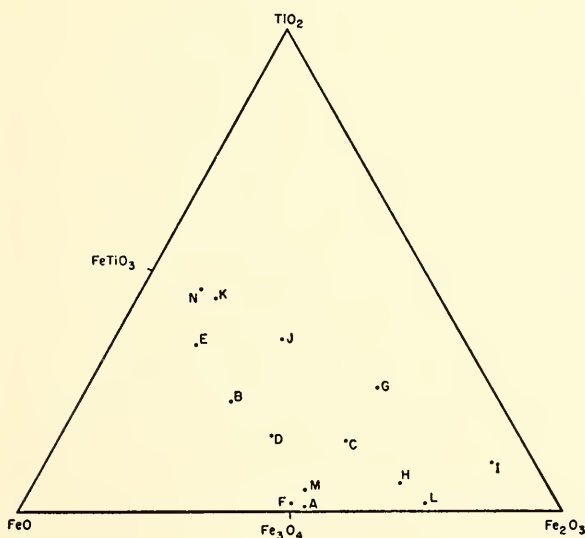


Fig. 4. Composition of the rock samples.

siderable, or little, magnetic interaction of the different species on one another. At the time of formation, some ferromagnetic minerals may appear as metastable single

caution, however, in accepting rock-magnetism data, of the sort usually presented, as evidence bearing satisfactorily on major geophysical phenomena.

#### MINERAL AGE MEASUREMENTS

*L. T. Aldrich, G. W. Wetherill, G. L. Davis,<sup>1</sup>  
and G. R. Tilton<sup>1</sup>*

About ten years ago, improvements in mass-spectrometric techniques and the development of chemical analysis by isotope dilution made it possible to measure the small quantities of radiogenic daughter products in ordinary rock-forming minerals. It thus appeared possible to extend greatly the scope of mineral age measurements, which had previously been limited to rocks containing uranium and thorium. Accordingly work was initiated in a number of laboratories with particular attention to the measurement of radiogenic argon in potassium minerals, and radiogenic lead in minerals like zircon that contain only small quantities of uranium.

In 1950 a mineral age program was started in this laboratory. The emphasis during the first few years was on the development and quantitative field testing of a mineral age method based on the decay of rubidium into strontium as well as the extension of the earlier work with zircons. In 1954 potassium-argon measurements began.

Until the last year workers in this field have been primarily concerned with the following three problems: the development of satisfactory chemical techniques for analysis of microgram quantities of the parent and daughter elements; investigations of the extent to which minerals have formed closed chemical systems with respect to the parent and daughter elements; the accurate determination of the decay constants for the decay of the parent into the daughter isotopes. Contributions made by this group on these problems are summarized in previous annual reports.

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Advances toward their solution have been substantial during the past several years, and although many questions remain unanswered, emphasis has now shifted to the application of the techniques to problems of geology. The shift in emphasis is reflected in the present report. Whereas in previous years most of the discussion has concerned the problems enumerated above, a large part of this report is devoted to actual geological applications. On the other hand, concern with the reliability of the techniques has not ended. As was stated last year, agreement of the

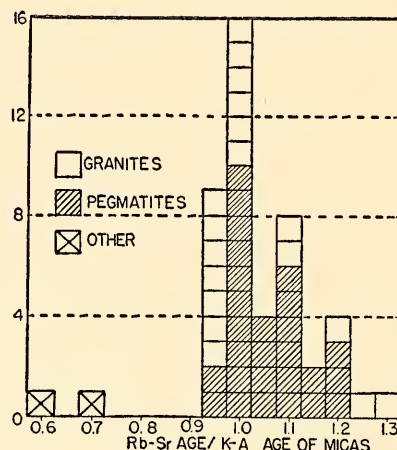


Fig. 5. Histogram showing ratio of K-A age to Rb-Sr age of all the mica samples for which this comparison has been made.

rubidium-strontium and potassium-argon ages for a sample of mica is a good indication that the mineral has formed a closed system. Therefore an age measurement is not considered satisfactory unless such agreement is found. The results of further comparisons of K-A and Rb-Sr ages of mica are shown in figure 5. Agreement between these two ages is most common, although not invariable. Attention is being given to those cases in which agreement is not found in order to see whether the lack of agreement is related to the condition or type of rock or to the history of a particular geographical region.

It was reported last year that satisfactory agreement between K-A ages of mica and concordant U-Pb ages of uraninite could be obtained if the specific gamma activity



of potassium were taken to be  $3.2 \text{ } \gamma/\text{g}/\text{sec}$ . During the past year, an absolute counting experiment has been carried out here which indicates that the specific gamma activity is slightly higher than this,  $3.39 \pm 0.12 \text{ } \gamma/\text{g}/\text{sec}$ . Thus even the best micas may lose about 5 per cent of their argon. The experimental errors are such, however, that the specific activities determined by the counting experiment and by the geological comparisons are not in disagreement.

The number of important geological problems that are amenable to study by these techniques is overwhelming, and workers in other laboratories as well as here have now turned their attention toward them. During the past year our group has begun an investigation of regional regularities in the ages of the Precambrian rock exposures. That such regularities probably exist was suggested by the remarkable agreement found between ages of rocks from various parts of the Grenville subprovince of the Canadian Shield. Early work by Ellsworth and subsequent work by other investigators has demonstrated that over this area of approximately 10,000 square miles all the rocks have an age of  $1000 \pm 100$  million years. Similarly in the Appalachian system almost all the igneous and metamorphic rocks had been found to have an age of  $300 \pm 100$  million years. These results suggest the hypothesis that throughout earth history there have been periods of a few hundred million years' duration in which extensive igneous and metamorphic activity took place in a given area. The techniques of age measurement should make it possible to discover the time and places of these orogenic events and to see whether any regularities can be found in their occurrence. As an early result of this investigation it has been found that, over a large part of Arizona, New Mexico, Colorado, and Wyoming, rocks were formed  $1350 \pm 100$  million years ago, and that in Ontario there is a large group of rocks 2600 million years of age. Combination of these results with those found in other laboratories indicates that there

is a long band of approximately 2600-million-year-old rocks extending from Wyoming through Montana, Minnesota, Manitoba, and Ontario into Quebec. Similar areas of very ancient rocks have been found in Africa and Australia. Less complete data indicate similar regional episodes elsewhere.

These results will be discussed in more detail in the following sections.

### *The Specific Gamma Activity of Natural Potassium*

Until recently one of the principal difficulties in the determination of K-A ages has been the lack of an accurate value for the decay constant of  $\text{K}^{40}$  for electron capture to  $\text{A}^{40}$ , or its equivalent, the specific gamma activity of potassium. In last year's annual report it was shown that good agreement was obtained between K-A ages of mica and concordant U-Pb ages of uraninite if a specific gamma activity of  $3.2 \text{ } \gamma/\text{g}/\text{sec}$  were used. It was pointed out, however, that probably the true specific gamma activity was slightly higher than this and that a few per cent of the radiogenic argon has been lost by the mica. To resolve this question an absolute counting experiment has been carried out which eliminates several of the uncertainties present in earlier measurements.

With the exception of the recent experiment of McNair, Glover, and Wilson (*Phil. Mag.*, 1, 199 [1956]), all previous determinations of the specific gamma activity have made use of counting techniques that did not distinguish between the ionization produced by the particular gamma ray being studied and other gammas or sources of ionizing radiation. The difficulty is not serious for  $\text{K}^{40}$  because of the simplicity of its gamma spectrum. But to know the efficiency of the counter for gamma rays of this energy (1.46 Mev) requires calibration of the counter with gammas from sources of known specific activity. Unfortunately, all the standard sources for this energy region either have more than one gamma ray in their spec-



trum or have a serious uncertainty in the fraction of the disintegrations that involve the emission of the standard gamma ray. These difficulties are sufficiently great to account easily for the wide spread in the results of earlier experiments.

In the measurement of McNair, Glover, and Wilson, referred to above, some of the difficulties were eliminated by means of a NaI(Tl) scintillation spectrometer which enabled them to use  $\text{Na}^{24}$  as a standard since they could single out those counts due to the 1.38-Mev gamma ray and reject those due to the 2.75-Mev gamma ray. In this way they obtained a value of 3.33  $\gamma/\text{g}/\text{sec}$  for the specific gamma activity. Although this experiment is superior to the earlier ones that did not make use of a scintillation spectrometer, two objections can be raised against it: the uncertainty in guessing the difference in the efficiencies of the counter at 1.38 and 1.46 Mev; and the fact that their experiment was designed to determine not the specific gamma activity but the  $\beta$ - $\gamma$  branching ratio. Since it is the specific gamma activity that is important for age measurements, their value of the branching ratio must be multiplied by the specific beta activity, thus introducing another serious probable error.

The specific gamma activity was determined here with a scintillation spectrometer;  $\text{Co}^{60}$  was used as a standard as well as  $\text{Na}^{24}$ , the efficiency of the counter being thus determined at 1.17, 1.33, and 1.38 Mev. The absolute activities of the standards were known as a result of absolute beta measurements made at the National Bureau of Standards by H. M. Seliger.

A diagram of the counting apparatus is shown in figure 6. A concentrated solution of potassium acetate was placed in the counting bottle above the 3 by 3 inch NaI(Tl) crystal. A single-channel pulse-height analyzer was used to obtain the spectrum shown in figure 7. The peak in the spectrum, known as the "photopeak," represents all the 1.46-Mev gamma rays that lose all their energy in the crystal. The 1.46-Mev gamma ray was counted by

setting the bias of the pulse-height analyzer so that all pulses of higher voltage were counted. The background was determined

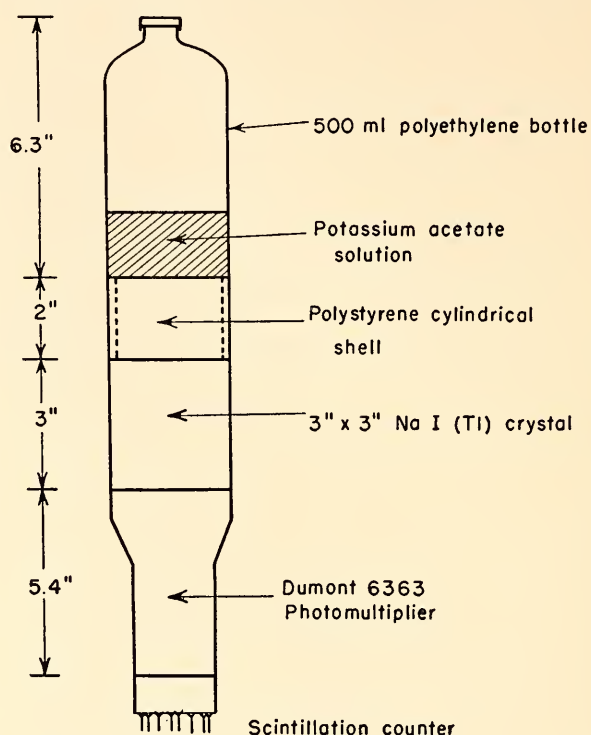


Fig. 6. Scintillation counter for the measurement of the specific gamma activity of potassium.

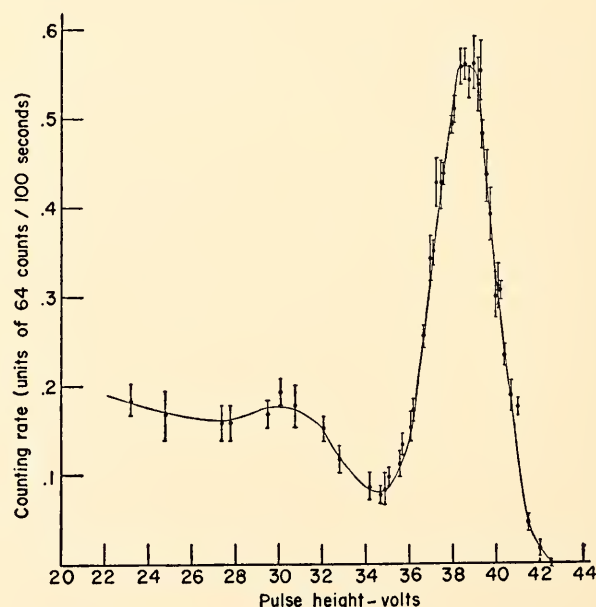


Fig. 7. Gamma-ray spectrum of  $\text{K}^{40}$ . The large peak on the right is the photopeak caused by those gamma rays that have lost all their energy in the NaI crystal.

in the same way with water instead of potassium acetate in the counting bottle.

The efficiency of the counter was meas-

ured by mixing standard  $\text{Na}^{24}$  and  $\text{Co}^{60}$  sources of relatively high specific activity in with the potassium acetate. The spectra

energy region from 35 to 40 volts and then using the spectrum, figure 8, to make corrections for the small part of the photo-

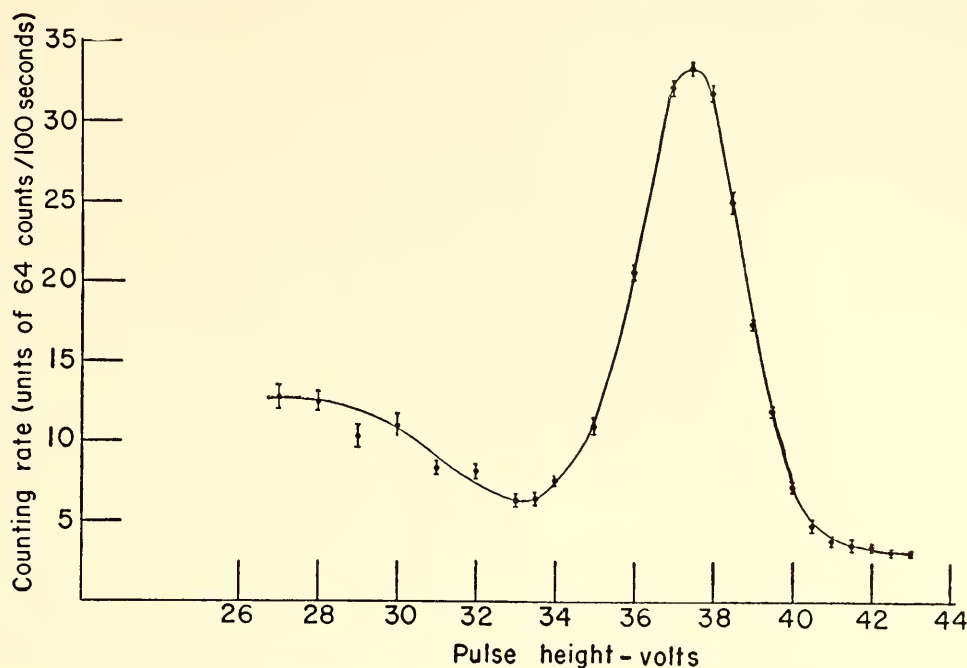


Fig. 8. Gamma-ray spectrum of  $\text{Na}^{24}$  showing the photopeak produced by the 1.38-Mev gamma ray.

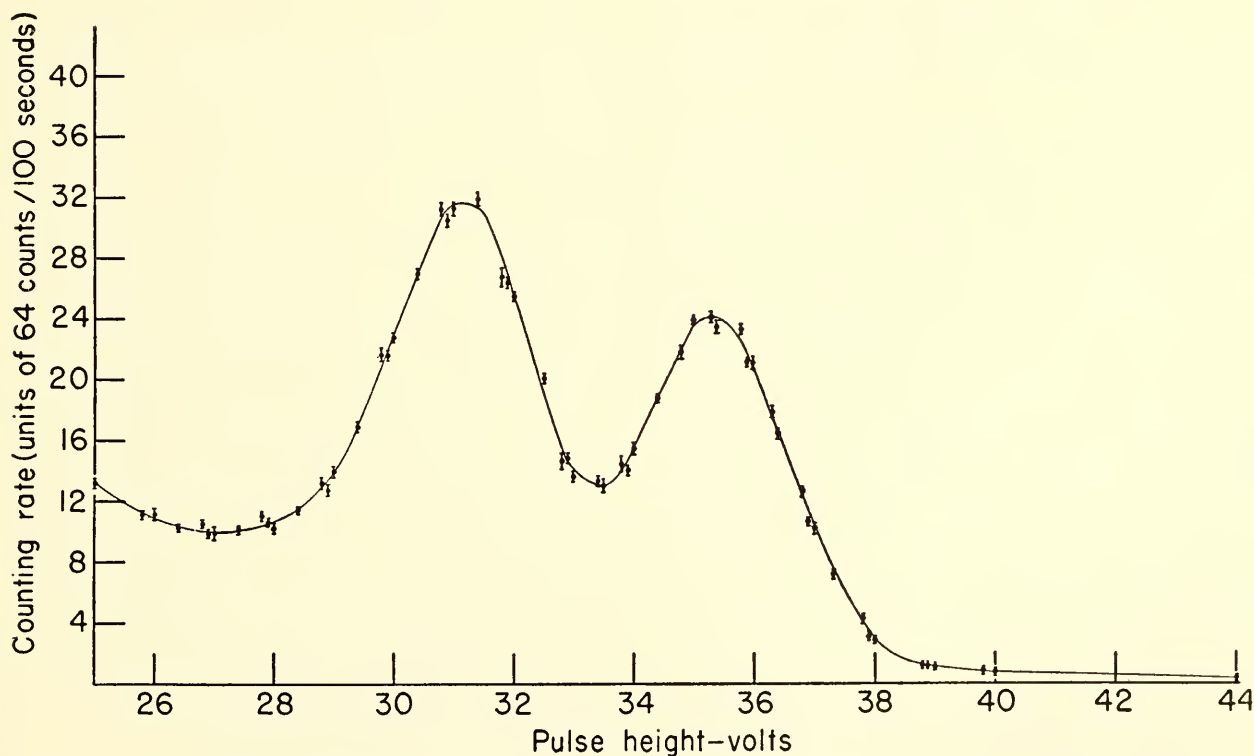


Fig. 9. Gamma-ray spectrum of  $\text{Co}^{60}$  showing the photopeaks produced by the 1.17- and 1.33-Mev gamma rays.

obtained with these sources are shown in figures 8 and 9. The photoefficiency of the 1.38-Mev gamma ray from  $\text{Na}^{24}$  was determined by counting all the pulses in the

peak that does not occur in this interval. It should be emphasized that the photoefficiency does not depend in first order on the accuracy of the gamma-ray spectrum, but

on the counting rate for the 35 to 40 volt interval. Similar measurements were made with  $\text{Co}^{60}$ . The two photopeaks were counted by counting the intervals 28 to 34 and 34 to 40 volts, respectively. The corrections were greater for  $\text{Co}^{60}$  because of the incomplete resolution of the two photopeaks. Corrections were made for the portion of the 1.33-Mev peak that lay below 34 volts by assuming that the 1.33-Mev  $\text{Co}^{60}$  peak has the same shape as the 1.38-Mev  $\text{Na}^{24}$  peak. Subtracting out the 1.33-Mev peak corrected in this way from the measured  $\text{Co}^{60}$  spectrum gives the 1.17-Mev peak. A check was made by comparing the calculated 1.17-Mev peak with the photopeak of the 1.12-Mev gamma ray from  $\text{Zn}^{65}$ . The shape of the two peaks was identical within experimental error. Although these corrections were rather tedious, the accuracy of the  $\text{Co}^{60}$  photoefficiencies is comparable to that of the  $\text{Na}^{24}$  photoefficiency. For both  $\text{Na}^{24}$  and  $\text{Co}^{60}$ , small additional corrections were made for coincidence between the two gamma rays.

The calculated efficiencies at the three energies 1.17, 1.33, and 1.38 Mev are plotted in figure 10. The curve through these three points is extrapolated to 1.46 Mev. The efficiency calculated in this way was then combined with the potassium integral counting data, and the specific gamma activity was found to be  $3.39 \pm 0.12$   $\gamma/\text{g}/\text{sec}$ . This value is in agreement with the result of McNair, Glover, and Wilson ( $3.33 \pm 0.15$   $\gamma/\text{g}/\text{sec}$ ), and is about 5 per cent higher than that given last year from measurements of the radiogenic argon content of mica samples of known age.

#### *The Retention of Argon by Minerals*

A number of analyses have been made of potassium minerals whose age is known from concordant U-Pb ages of cogenetic minerals. By means of the specific gamma activity of 3.39  $\gamma/\text{g}/\text{sec}$ , the ratio of  $\text{A}^{40}$  to  $\text{K}^{40}$  that would be found in the potassium mineral if all the radiogenic argon had been retained can be calculated. Comparison of this ratio with that found by

analysis of the potassium mineral indicates the fraction of the radiogenic argon retained by the mineral. Previously reported measurements made by this group have been combined with measurements made at the University of Chicago by Wasserburg and Hayden to calculate the retentivities shown in table 1. Adequate data are available only for mica and feldspar at present.

The retentivity of mica averages about 90 per cent; that of feldspar is considerably lower. The error in these retentivity

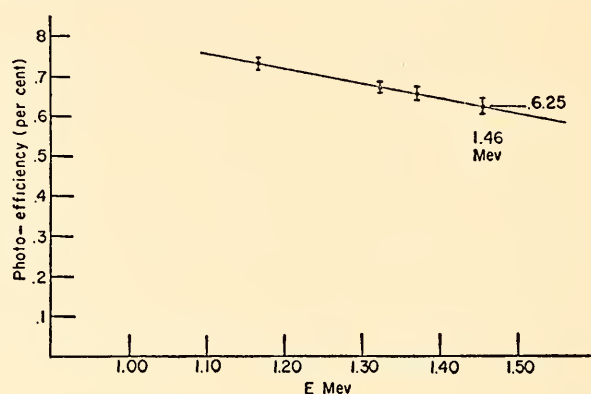


Fig. 10. The measured efficiency of the NaI crystal as a function of energy. The curve is extrapolated to 1.46 Mev to determine the efficiency for the  $\text{K}^{40}$  gamma ray.

measurements is about 10 per cent, owing to the uncertainties in the true value of the specific gamma activity, the comparison U-Pb ages, and the analytic determination of potassium and argon. Thus it is difficult to be absolutely certain that the average mica sample loses any argon; the data indicate, however, that micas probably lose a small fraction of their radiogenic argon whereas feldspars lose about 25 per cent. The mica ages in this report are calculated using a specific gamma activity of 3.24  $\gamma/\text{g}/\text{sec}$ , which is approximately equivalent to using 3.39  $\gamma/\text{g}/\text{sec}$  with a 5 per cent correction for argon loss.

There are other minerals that contain small amounts of potassium, and with modern analytical techniques it should be possible to measure their radiogenic argon content and hence their retentivity. It may be that minerals will be found that retain a greater fraction of their argon than the micas.



TABLE 1. Retention of Argon by Minerals as Indicated by Comparison with Concordant Uraninite Ages

Specific gamma activity of 3.39  $\gamma$ /g/sec and specific beta activity of 27.6  $\beta$ /g/sec  
(Many of these comparisons are obtained from the work of Wasserburg and Hayden, *Geochim. et Cosmochim. Acta*, 7, 51 [1954]; 9, 153 [1956].)

Sample Location	Concordant Uranium Age, million years	Argon Reten-tivity, %
A. Micas		
1. Portland, Conn. ....	267	0.95
2. Glastonbury, Conn. ....	255	.97
3. Spruce Pine, N. C. ....	375	.88
4. Branchville, Conn. ....	367	.99
5. Parry Sound, Ont. ....	994	.93
6. Cardiff Twp., Ont. ....	1020	.92
7. Wilberforce, Ont. ....	1030	.87
8. Keystone, S. Dak., lepidolite .....	1600	.81
9. Keystone, S. Dak., muscovite .....	1600	.95
10. Viking Lake, Sask. ....	1890	.93
11. Bikita, S. Rhodesia ....	2650	.80
B. Feldspars		
1. Portland, Conn. ....	267	.77
2. Glastonbury, Conn. ....	255	.81
3. New Bedford, N. Y. ....	355	.77
4. Branchville, Conn. ....	367	.75
5. Parry Sound, Ont., microcline 1 .....	994	.72
6. Parry Sound, Ont., microcline 2 .....	994	.78
7. Parry Sound, Ont., albite..	994	.60
8. Cardiff Twp., Ont., sample 1 .....	1010	.81
9. Cardiff Twp., Ont., sample 2 .....	1010	.76
10. Wilberforce, Ont. ....	1030	.77
11. Tory Hill, Ont., pegmatite .....	1030	.68
12. Tory Hill, Ont., granite...	1030	.68
13. Keystone, S. Dak. ....	1600	.59
14. Viking Lake, Sask. ....	1890	.79

*Ages of Rocks in the Canadian Shield*

For a number of years it has been recognized that the pegmatites intruding the older rocks in the Grenville subprovince are approximately 1000 million years of age. More recently, measurements of ages

of igneous and metamorphic rocks in this area have shown that the age of the igneous intrusion and the metamorphism is likewise 1000 million years. Rocks of this age have also been found south of the Grenville region in New York State, both in the Adirondacks and in the Catskill Mountains. Measurements of "Grenville ages" made at the Carnegie Institution are shown in table 2, and the location of the Canadian samples on the map (fig. 11). Numerous similar results have been found in other laboratories, but the boundaries

TABLE 2. Rocks of Grenville Age

Sample Location	Age, million years			
	K-A	Rb-Sr	U <sup>238</sup> -Pb <sup>206</sup>	U <sup>235</sup> -Pb <sup>207</sup>
1. Wilberforce, Ont..	975	1000	1040	1050
2. Cardiff Twp., Ont. ....	1010	1030	1020	1020
3. Bancroft, Ont. ...	890	990		
4. Wavy Lake, Ont..	1025	1075		
5. Canada Hill gneiss, Bear Mt., N. Y..	930	1030	1020	1060
6. Storm King granite, Bear Mt., N. Y. ....	900			
7. Natural Bridge, N. Y. ....			1025	1065

of this "Grenville orogeny" have not yet been established.

Farther to the north in Ontario, much more ancient rocks have been found, all approximately 2600 million years of age, representing the oldest rocks that have been found in North America. The data are shown in table 3. As a result of measurements made here and elsewhere, there appears to be a belt of these very old rocks extending from Wyoming through Montana, Minnesota, Manitoba, and Ontario into Quebec. In eastern Ontario near the Quebec border these rocks appear within about 150 miles of the Grenville rocks, and later measurements of the rocks around Lake Timiskaming in Ontario and Quebec may bring these rocks of such different age into even closer contact.

A number of measurements have also

been made on rocks lying in the region between these two areas. Here the K-A and Rb-Sr ages are generally in disagreement, in contrast to the other two areas discussed, and also in contrast to our previous experience. Some of the discordance may result from the heating and partial recrystallization of the 2600-million-year-old rocks during the Grenville orogeny,

but it also seems probable that rocks of intermediate age occur in this region. The results of these measurements are found in tables 4, 5, and 6.

The Cutler batholith (table 4) intrudes the Sudbury series, and thus these sedimentary rocks are older than 1350 million years. The agreement of the Rb-Sr ages of both mica and feldspar from the peg-



Fig. 11. Map showing the location of samples from the Canadian Shield.

TABLE 3. Rocks Approximately 2600 Million Years of Age

Location and Sample	Age, million years	
	Rb-Sr	K-A
Hearst, Ont., pegmatite.....	2605	2595
Kirkland Lake, Ont., Round Lake lamprophyre .....	2600	2450
Kirkland Lake, Ont., Round Lake granite .....	2640	2530
Timmins, Ont., granite.....	2470	2520
Silver Leaf Mine, S. E. Manitoba..	2640	2210

matites and the agreement of the K-A ages of the micas and the Rb-Sr age of the granite suggest that the batholith was formed about 1750 million years ago and reheated 1350 million years ago. The data are insufficient to establish these events, however.

The Copper Cliff (table 5) rhyolite is a highly metamorphosed rock; originally it was probably an intrusive igneous rock, although some workers have thought it to be a lava or a sedimentary rock. The

Rb-Sr and K-A ages are the most discordant ones that have been found. In particular, the K-A age of the mica is much higher than the Rb-Sr age.

Other ages found in this region are shown in table 6. Here are two additional K-A ages appreciably higher than Rb-Sr ages. The Sudbury breccia contains pieces

TABLE 4. Age Determinations on the Cutler Batholith

Sample		Age, million years	
		Rb-Sr	K-A
Pegmatite 1	Muscovite . . . . .	1750	1440
	Feldspar . . . . .	1760	1165
Pegmatite 2	Muscovite . . . . .	1700	1420
Granite	Biotite . . . . .	1325	1380

TABLE 5. Age Determinations on the Copper Cliff Rhyolite

Sample		Age, million years	
		Rb-Sr	K-A
Muscovite . . . . .		1730	1390
Biotite . . . . .		1220	2130
Feldspar . . . . .		2360	1400

TABLE 6. Other Age Determinations in the Sudbury Area

Location and Sample	Age, million years	
	Rb-Sr	K-A
Sudbury, gabbro . . . . .	1325	1830
Sudbury, breccia (matrix) . . . .	1440	1870
Levack, norite . . . . .	1830	
Cobalt, Ont., lamprophyre . . . . .	2050	2160

of the Copper Cliff rhyolite and the Mc-Kim formation, and hence these rocks are older than the mica in the matrix of the breccia. Although the Rb-Sr and K-A ages are discordant there is little doubt that the breccia is older than 1500 million years, and hence the rocks found in the breccia cannot be late Precambrian. The Sudbury gabbro intrudes the Missasagi quartzite at Sudbury. As a result of the age measurement it is found that this rock is older than

the Grenville rocks. Thus all the rocks in this region appear to be intermediate in age between the 1000-million-year-old rocks in the Grenville subprovince and the 2600-million-year-old rocks farther north, but no conclusions can yet be drawn about their exact ages and relationships. These results illustrate the importance of measuring both the Rb-Sr and the K-A ages of a mica sample.

TABLE 7. Potassium-Argon and Rubidium-Strontium Ages of Rocks from Western United States

K-A ages are calculated from decay constants of  $K^{40}$  of  $\lambda_e=0.557 \times 10^{-10} \text{ yr}^{-1}$ ,  $\lambda_\beta=4.72 \times 10^{-10} \text{ yr}^{-1}$  or a total half-life of  $K^{40}$  of  $1.31 \times 10^9$  years. Rb-Sr ages are calculated using a half-life for  $Rb^{87}$  of  $50 \times 10^9$  years.

Sample Location	Age, million years	
	K-A	Rb-Sr
1. Gneiss, Zoroaster, Grand Canyon, Ariz. . . . .	1390	1370
2a. Lawler Peak granite, Bagdad, Ariz. . . . .	1410	1390
2b. Pegmatite in Lawler Peak granite . . . . .	1410	1500
3. Pegmatite, Wickenburg, Ariz..	1160	1300
4. Pidlite Mine, Mora Co., N. M..	1330	1490
5. Granite, Sandia Mts., Albuquerque, N. Mex. . . . .	1350	1340
6. Harding Mine, Dixon, N. Mex..	1300	1300
7. Uncompahgre granite, Mesa Co., Colo. . . . .	1320	1320
8. Granite, Doyleville, Colo. . . . .	1320	1310
9. Brown Derby pegmatite, Ohio City, Colo. . . . .	1330	1420
10. Granite, Sherman, Wyo. . . . .	1420	1410

Another group of granitic rocks from western United States present interesting analyses. The ages of micas from these rocks are given in table 7, and the locations of the rocks are shown in figure 12. The sample numbers in the table correspond to the numbered locations on the map. Four other locations are also shown on the map. The age of the sample from each of these locations is indicated by letter, *A* corresponding to 2500, *B* to 1600, and *C* to 1100



million years. The map shows all the Precambrian rocks measured at this laboratory from these states.

The agreement of the ages in table 7 shows that the mica in all these rocks was formed close to 1350 million years ago, and, therefore, that there was widespread crystallization of granitic rocks at that time in

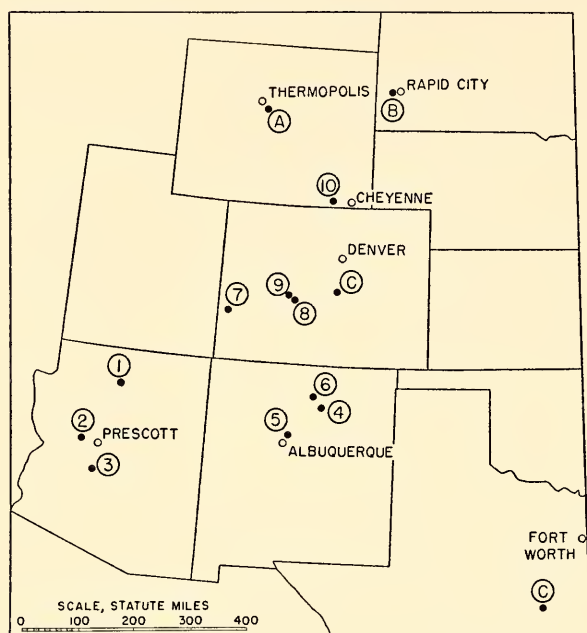


Fig. 12. Map showing the location of samples from western United States.

this area. It is suggested that this crystallization accompanied a period of regional rock formation comparable to the period of igneous intrusion and metamorphism in the Grenville subprovince. It is also evident that these mica ages have been preserved in spite of subsequent geologic events in western United States such as the Laramide orogeny.

Table 8 gives the ages of several South African micas for which both K-A and Rb-Sr ages have been measured. Samples from Manano and Karema were provided by Dr. L. Cahen, of the Belgian Congo Museum. The Muika sample was part of the collection analyzed by L. O. Nicolaysen several years ago here at the Carnegie Institution. From geologic evidence it was thought that the Karema pegmatite was formed as part of a much earlier process

TABLE 8. Ages of African Rocks

Sample Location	Age, million years	
	K-A	Rb-Sr
Muika, Belgian Congo.....	950	950
Manano, Belgian Congo.....	870	975
Karema, Tanganyika Territory..	980	1100
Bikita, S. Rhodesia.....	2450	2680
Kubuta, Swaziland .....	2730	2920

than that which formed the Muika and Manano pegmatites. These measurements show that, if this inference is true, the later process completely removed any trace of the earlier history of the mica from the Karema pegmatite. The analytical data on the Muika mica concurred with those obtained by Nicolaysen within 3 per cent.

The Bikita and Kubuta micas were also part of the Nicolaysen collection. The Bikita ages have been presented before, but are included for comparison with the Kubuta ages. The Kubuta ages are the greatest obtained in our laboratory by either the Rb-Sr or the K-A method.

## THEORETICAL AND STATISTICAL GEOPHYSICS

*S. E. Forbush*

### *EQUATORIAL ELECTROJET*

Near the magnetic equator the amplitude,  $A_H$ , of the diurnal variation in the horizontal component,  $H$ , of the geomagnetic variations is unusually large. At Huancayo, Peru, the abnormality exceeds that observed elsewhere. Along the west coast of South America,  $A_H$  was observed

in 1949 at fourteen stations by A. A. Giesecke, Jr., Director of the Instituto Geofísico de Huancayo. These stations extended from geographic latitude  $3.4^\circ$  N to  $16.2^\circ$  S. The observed rapid decrease of  $A_H$  with distance north or south of the magnetic equator indicated a concentrated narrow band of electric current flowing

eastward in the upper atmosphere during midday. This concentrated current, known as the equatorial electrojet, is superposed on the more diffuse current distribution that accounts for the normal quiet-day magnetic diurnal variation,  $S_q$ .

The existence of the equatorial electrojet effect on the quiet-day diurnal variation near the magnetic equator is thus definitely established. To determine whether similar electrojet effects occur for other variations in the geomagnetic field, and if so to determine whether the same electrojet is involved, a chain of four temporary magnetic recording stations is now being established for the International Geophysical Year on the west coast of Peru, to operate in conjunction with the John A. Fleming Observatory of the Instituto Geofísico de Huancayo. At each of the four temporary IGY stations a portable Askania variograph will register the variations in declination, horizontal intensity, and vertical intensity. The project is made possible through grants approved by the Geomagnetic Panel of the U. S. International Geophysical Year and through the co-operation of the U. S. Coast and Geodetic Survey, which has lent the four variographs to the Department of Terrestrial Magnetism, and the help of the Instituto Geofísico de Huancayo, which has co-operated in the preliminary survey and will manage the operation of the five stations.

The preliminary survey, using two variographs, involved two or three days' continuous registration of the variations in the geomagnetic field at each of some sixteen stations. This survey was begun in March and completed in May 1957. The results provided a necessary guide to locate the final stations, and indicated an electrojet with total extension of roughly 500 miles in the north-south direction. Moreover, these observations also provided data from which to calculate the height of the electrojet, previously undetermined since, near the magnetic equator, the variation with latitude of the diurnal variation of declina-

tion and of the vertical field had not been measured.

Continuous operation of the four variographs during the IGY should provide data from which to determine whether electrojet effects occur in the lunar diurnal variation, magnetic sudden commencements, solar-flare effects, magnetic storm changes ( $D_{st}$ ), etc. If it turns out that no electrojet effects are found for changes during magnetic storms, then the records of vertical intensity from the two stations nearest the magnetic equator promise to provide a measure for the amplitude of the quiet-day diurnal variation and its variability, which should be free of the effects of magnetic disturbance that often spoils Bartels' present measure ( $\delta w$ ), based on the diurnal variation of  $H$  at Huancayo.

#### VARIABILITY OF GEOMAGNETIC DIURNAL VARIATION AND OF IONOSPHERIC WINDS

Last year's report described the results of a statistical experiment showing that the magnitude of the solar flare, or *crochet*, effect in the horizontal magnetic component at Huancayo was definitely greater, on the average, on days when the quiet-day diurnal variation,  $S_q$ , in the geomagnetic field was greater. Although the amplitude of  $S_q$  is known to increase with sunspot number, the size of *crochets* was found to be independent of sunspot number; thus the above relation between *crochet* size and amplitude of  $S_q$  was not due to a solar activity effect common to both. It was concluded that both effects could be ascribed to variations in the strength of the wind system that drives the dynamo responsible for  $S_q$ , since on days with stronger wind systems both the size of *crochets* and the amplitude of  $S_q$  would be larger.

In the last few years ionospheric wind velocities have been measured from the rate of drift of meteor trails by Professor A. C. B. Lovell and his colleagues at Jodrell Bank. Their results showed that the semi-diurnal variation of wind velocity, in the range of heights where it was measured,



had the wrong phase, according to the dynamo theory, to explain the quiet-day magnetic variation. The results obtained also showed large variations from day to day in the amplitude of the semidiurnal wind components (N-S and E-W) and in the daily average wind velocity in the N-S and E-W directions. Professor Lovell has made available to us the amplitudes of the semidiurnal wind component for 70 days together with the daily values of the prevailing wind components. Arrangements have been made to obtain constants for recent magnetograms from Huancayo which will be measured with a recently constructed rapid scaling device to furnish material from which to derive the amplitude of  $S_q$ . It will then be possible to determine statistically whether the measured variability of the ionospheric winds is correlated with the variability of the amplitude of  $S_q$ .

#### COSMIC-RAY INVESTIGATIONS

*Twenty-seven-day variation in cosmic-ray intensity and in the geomagnetic field.* The phase and amplitude of the 27-day waves in horizontal magnetic intensity,  $H$ , at Huancayo have been determined for about 155 solar rotations. Magnetic results are expected shortly from Huancayo for some of the years after 1947; these will be analyzed for the 27-day waves. With results already at hand for the 27-day waves in cosmic-ray intensity for 250 solar rotations it will thus be possible to determine rather reliably whether there is any significant phase difference between the maxima of the cosmic-ray waves and the minima of the waves in  $H$  at Huancayo. The results should have a significant bearing on theories for explaining decreases in cosmic-ray intensity associated with magnetic disturbance.

*Old cosmic-ray program.* Compton-Bennett meters were satisfactorily operated throughout the report year at Godhavn (Greenland), Climax (Colorado, U. S.), Ciudad Universitaria (Mexico, D. F.), Huancayo (Peru), and Christchurch (New

Zealand). The station at Cheltenham (Maryland, U. S.) was transferred in October 1956 to the Fredericksburg Magnetic Observatory at Fredericksburg (Virginia, U. S.). Tabulations of bihourly means of ionization corrected for bursts and barometric pressure for Huancayo from 1946 to 1955, and for Cheltenham from 1937 to 1954, as well as summaries for Godhavn and Christchurch have been published in volume XX of Carnegie Institution of Washington Publication 175. These results, compiled in collaboration with the Instituto Geofísico de Huancayo, The Danish Meteorological Office, The Department of Scientific and Industrial Research, New Zealand, and the U. S. Coast and Geodetic Survey, together with those contained in earlier volumes, make available to investigators many of the essential data obtained since the start of the Department's cosmic-ray program.

*Large ionization chamber.* The large cosmic-ray ionization chamber was maintained in essentially continuous operation at Derwood during the report year. No solar-flare effects have been observed since February 23, 1956.

*Co-operation in operation of cosmic-ray meters.* The successful operation of Compton-Bennett cosmic-ray meters over a long period at so many stations has been possible only through the wholehearted and unselfish co-operation of several organizations and individuals. We wish to express our appreciation to the following organizations for the operation and maintenance of cosmic-ray meters: The Danish Meteorological Institute and the staff of its Godhavn Magnetic Observatory at Godhavn, Greenland; the U. S. Coast and Geodetic Survey and the staff of its magnetic observatory at Cheltenham, Maryland (at Fredericksburg, Virginia, since October 1956); the High Altitude Observatory of the University of Colorado and its staff at Climax, Colorado; the Instituto Nacional de la Investigación Científica and the Universidad de Mexico, Mexico, D. F.; the Government of Peru and the staff of its



Instituto Geofísico de Huancayo for making available the Compton-Bennett records from Huancayo; and the Department of

Scientific and Industrial Research and the staff of its Magnetic Observatory at Christchurch, New Zealand.

## LABORATORY PHYSICS

### NUCLEAR PHYSICS

*N. P. Heydenburg, G. M. Temmer,<sup>1</sup> and G. F. Pieper*

During the past year, our continuing Coulomb excitation studies of nuclear energy levels included an investigation of the gamma radiation observed in krypton with enriched targets, further analysis of the gamma-ray spectrum of  $\text{Fe}^{57}$ , and a study of the rotational levels of the dysprosium isotopes with enriched targets. In addition to the Coulomb excitation work, we have been engaged in an experimental study of the angular distribution and angular correlation of the protons and gamma radiation emitted when fluorine is bombarded by 6- to 6.5-Mev alpha particles. The interest here is to determine, if possible, whether the reaction proceeds by compound-nucleus formation or by some direct-interaction process.

### COULOMB EXCITATION STUDIES

*Krypton.* In last year's annual report, tentative isotopic assignments were given for the four gamma rays observed from natural krypton bombarded by 6.4-Mev alpha particles. With six isotopes present in natural krypton, it was desirable to have these assignments checked with isotopically enriched samples.

During the year two gas samples of krypton with different isotopic enrichments were made available to us from Yale. In co-operation with C. E. Anderson, as a Guest Investigator from Yale, these samples have been bombarded here with 6.1- and 6.6-Mev alpha particles, using our high-voltage equipment. With three targets having different isotopic ratios it was possible to make the following gamma-ray

energy assignments unambiguously:  $\text{Kr}^{78}$ , 450 kev;  $\text{Kr}^{80}$ , 620 kev;  $\text{Kr}^{82}$ , 780 kev; and  $\text{Kr}^{84}$ , 880 kev. No gamma rays were observed in  $\text{Kr}^{83}$  and  $\text{Kr}^{86}$ . One of the targets, enriched 30 fold in  $\text{Kr}^{78}$  (0.35 per cent abundant in natural krypton), made it possible to observe the gamma ray associated with this isotope.

The gamma rays from these even-charge, even-mass isotopes are due to electric quadrupole excitation of the first spin  $2^+$  level. The energies of the  $2^+$  states increase in a systematic way with increasing mass of the krypton isotope, whereas the gamma-ray intensity decreases. According to nuclear shell theory,  $\text{Kr}^{86}$  has a closed shell system for neutrons (neutron number 50). Our results for the first-excited states of the krypton isotopes are consistent with the now well established trends of first-excited-state energies, which have much lower values for nuclei having partially filled shells and rise to quite large values at the closed shells. Similarly, the reduced transition probabilities  $B(E2)$  decrease in approaching a closed shell. Again we observe a strong excitation for  $\text{Kr}^{78}$  and have found no evidence of a gamma ray for  $\text{Kr}^{86}$ .

*Iron.* Natural iron has a high abundance of the isotope  $\text{Fe}^{56}$ , and the  $2^+$  first-excited state of this isotope at 854 kev is readily excited by 6-Mev alpha particles. Gamma rays at 123 kev and 350 kev are also seen; they are due to  $\text{Fe}^{57}$ , which has an abundance of only 2.2 per cent in natural iron. We have investigated the energy levels of  $\text{Fe}^{57}$  with a target enriched to 59 per cent. The gamma-ray spectrum observed with an alpha-particle energy of 4 Mev is shown in figure 13. In the present study we have been concerned with the properties of the third-excited level in  $\text{Fe}^{57}$ , since earlier investigations both here and by a group at Oxford had established the level scheme

<sup>1</sup> On leave of absence, 1956-1957, as Guggenheim Fellow, Saclay Laboratories, Paris, and Institute for Theoretical Physics, Copenhagen.

for the first and second levels. The level scheme and associated gamma-ray transitions are shown in figure 14. We have shown that the 350-keV gamma ray is due to the Coulomb excitation of a level at 365 keV rather than at 350 keV, by observations on the yield of this gamma ray as a function of the alpha-particle energy.

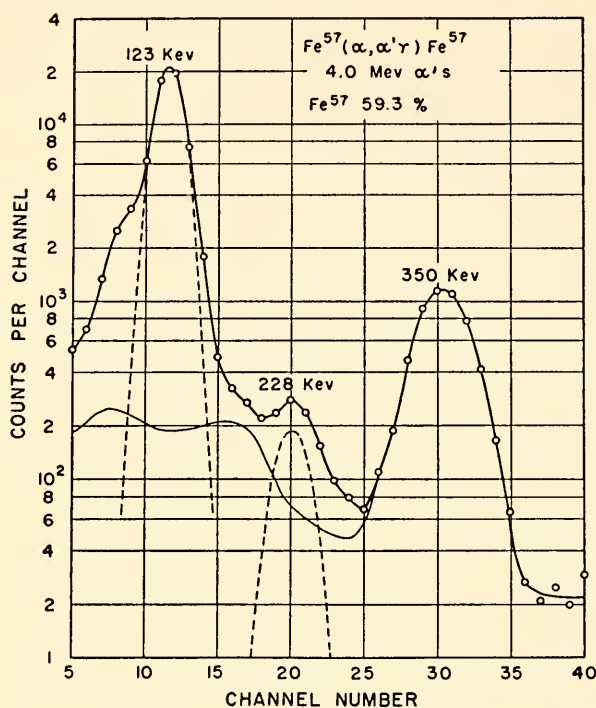


Fig. 13. Gamma-ray spectrum from the Coulomb excitation of  $\text{Fe}^{57}$  by 4-MeV alpha particles. The solid line connected to the peak at 350 keV is the shape of a single gamma ray of that energy, obtained under similar conditions from the Coulomb excitation of  $\text{Ru}^{104}$ . The dashed parabolas labeled 123 and 228 keV represent the photopeaks of gamma rays of these energies, after allowance has been made for higher-energy radiations and background. The "escape peak" from the 123-keV gamma ray is evident at about channel 9.

These results are shown in figure 15. The theoretical yield curves calculated from Coulomb excitation theory for the excitation of a 350-keV and a 365-keV level have been normalized to the experimental point at 4 MeV. The curve for  $\Delta E = 365$  keV is in better agreement with the experimental data. It should also be noticed that the 230-keV gamma-ray yield follows the curvature for  $\Delta E = 365$  keV. That the 230-keV gamma ray is also due to the excitation of

the 365-keV level and is a cascade transition to the 137-keV level was shown by observing coincidences between the 230-keV and the 123-keV gamma rays.

Since the ground-state spin of  $\text{Fe}^{57}$  is  $1/2$ , and only electric quadrupole ( $E2$ ) transitions are induced by Coulomb excitation, the 365-keV level can have spin of either  $3/2$  or  $5/2$ . From our results,  $5/2$  seems

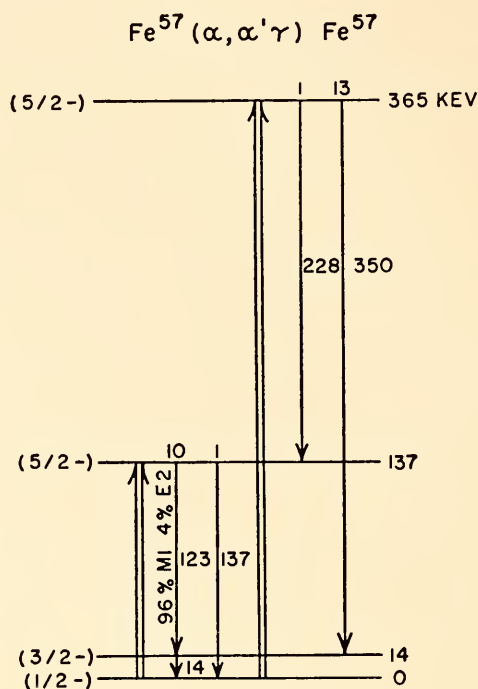


Fig. 14. Proposed level scheme for  $\text{Fe}^{57}$ .

more likely for the following reasons: the observed branching ratio for the 350-keV and the 230-keV gamma rays is in much better agreement with the predicted ratio for spin  $5/2$  than for  $3/2$ ; if the spin were  $3/2$ , a transition to the ground state by magnetic dipole ( $M1$ ) radiation would be more probable than to the first-excited state, whereas for  $5/2$  spin only  $E2$  radiation is allowed to the ground state, which should be much weaker than  $M1$  radiation to the first-excited state. We observe the transition predominantly to the first-excited state; a gamma ray of 365 keV cannot be present to an amount greater than 5 per cent of the 350-keV gamma ray.

Angular distributions were also observed for the 230-keV and 350-keV gamma rays. The theoretical predictions are not unique for either of the two spin choices, since



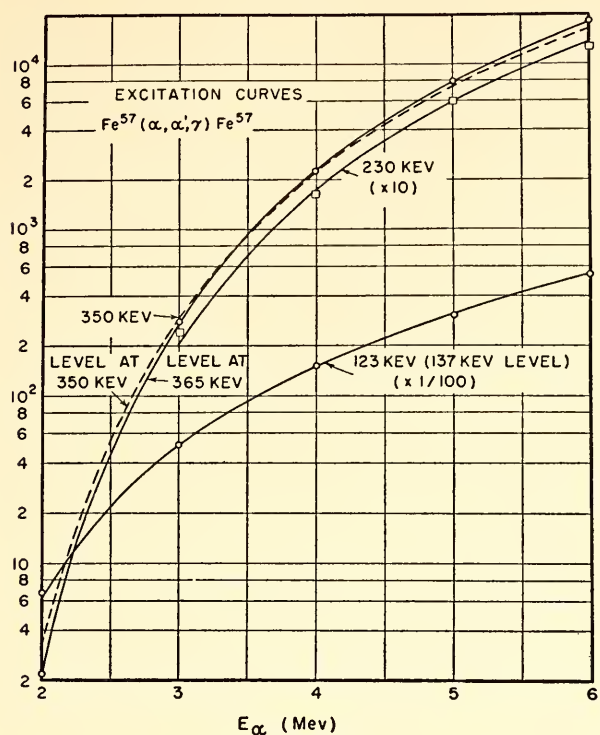


Fig. 15. Excitation curves for the Coulomb excitation of Fe<sup>57</sup> by alpha particles. The points are the experimental gamma-ray yields. The solid and dashed curves are the theoretical thick target E2 Coulomb excitation functions for the level positions indicated.

the Coulomb excitation of enriched isotopes of dysprosium, recently available from Oak Ridge. In each of the two even-even isotopes Dy<sup>162</sup> and Dy<sup>164</sup> we observed only one gamma ray, with energies 82 kev and 75 kev, respectively. These are due to the excitation of the first of the rotational levels in the sequence having spins 2<sup>+</sup>, 4<sup>+</sup>, 6<sup>+</sup> . . . with ground-state spin 0<sup>+</sup>. By E2 excitation we can excite only the first 2<sup>+</sup> level.

For odd-*A* nuclei it is possible to excite two of the rotational levels by E2 transitions since the levels have spins  $I_0+1$ ,  $I_0+2$ ,  $I_0+3$ , . . . , where  $I_0$  is the ground-state spin. These levels, according to the theory of Bohr and Mottelson, should have energies given by

$$E=\frac{\hbar^2}{2\mathfrak{I}}[I(I+1)-I_0(I_0+1)]$$

where  $I$  is the spin of the level, and  $\mathfrak{I}$  is the effective moment of inertia of the rotating nucleus.

TABLE 9. Experimental Results from the Coulomb Excitation of Fe<sup>57</sup>

Column 1 gives the energies of the levels excited in Fe<sup>57</sup>. Column 2 gives the reduced upward transition probability,  $B(E2)$ , in units of  $e^2 \times 10^{-48}$  cm<sup>4</sup>. Column 3 gives  $F$ , the favored factor, the ratio of the observed  $B(E2)$  to that expected for a single particle transition. Column 4 gives the energies of the observed gamma rays, and column 5 the branching ratios of the levels from which they originate. Columns 6 and 7 give  $\delta$  and  $\delta^2$ , where  $\delta^2$  is the ratio of E2 to M1 components in the radiation.

Fe <sup>57</sup> Level, kev	$B(E2)$	$F$	Gamma-Ray Energies, kev	Branching, per cent	$\delta$	$\delta^2 = \frac{E2}{M1}$
137	0.050	13	123	91	+0.19	0.04
			137	9	Pure E2	
365	0.033	8.4	228	7	-0.18	0.03
					+2.6	6.7
			350	93	+0.28	0.08
					-90	8000

another parameter,  $\delta^2$ , the mixing ratio of E2 to M1 radiation, is involved for the downward transitions. Our results for Fe<sup>57</sup> are summarized in table 9.

*Dysprosium.* Continuing our investigations of the rotational bands that occur in the region of the rare-earth nuclei, we have observed the gamma rays resulting from

In our earlier survey of the rare-earth nuclei we had observed two gamma rays in natural dysprosium, one of which at 166 kev was thought to be due to the second rotational level in one or both of the odd-*A* isotopes Dy<sup>161</sup> and Dy<sup>163</sup>. Targets enriched to 75 per cent were available for these two isotopes. This enrichment proved



to be satisfactory for  $\text{Dy}^{163}$ . The 166-keV gamma ray was found to be due entirely to this isotope. Another gamma-ray peak at about 75 keV was found to be due in part to  $\text{Dy}^{163}$  and in part to the neighboring isotopes  $\text{Dy}^{162}$  and  $\text{Dy}^{164}$ . The two gamma rays in  $\text{Dy}^{163}$  at 75 keV and 166 keV are believed to be due to excitation of the first and second rotational levels (of the ground-state rotational band). The ratio of the energy of the second-excited level to that of the first-excited level agrees within our experimental errors to the predicted ratio calculated from the equation above for  $I_0=5/2$ , which is the known ground-state spin of  $\text{Dy}^{163}$ . A much weaker gamma ray at about 93 keV was observed which could correspond to a transition from the second- to the first-excited rotational levels. This interpretation was confirmed by observing coincidences between the 93-keV and 75-keV gamma rays.

The situation in  $\text{Dy}^{161}$  was not so simple, however; at first sight there did not appear to be any gamma rays associated with this isotope, but a careful analysis did reveal a weak gamma ray at about 103 keV. The strong gamma ray observed at 82 keV was accounted for by the presence of the neighboring isotope  $\text{Dy}^{162}$  in the sample. Further, the  $K$  X-ray peak which is always excited in these rare-earth targets appeared to be stronger than would have been expected from observations on the  $\text{Dy}^{162}$  target. It is known that the  $K$  X-ray peak results primarily (for excitation by alpha particles) from a so-called fluorescence process. In this process, for example in  $\text{Dy}^{162}$ , the 82-keV transition can occur both by gamma emission and by conversion electron emission. In the latter case, for  $K$  conversion, a  $K$  electron is emitted, leaving a vacancy in the  $K$  shell which can be filled from a higher shell with emission of a  $K$  X-ray. Hence it would be expected that the  $K$  X-ray peak should follow the same yield as a function of alpha energy as the 82-keV gamma. This was found to be true for the  $\text{Dy}^{162}$  target but not for

$\text{Dy}^{161}$ . The yield of the peak, having about the same energy as the  $K$  X-ray peak, varied more slowly with energy than in the  $\text{Dy}^{162}$  target, and indeed it followed a theoretical yield curve for the excitation of a 46-keV gamma ray. Hence, it seems reasonable to believe that most of this peak is due to a gamma ray of about 46-keV energy. We have suggested that this ray is due to the excitation of the first rotational level and that the weak gamma ray at 103 keV is due to the excitation of the second rotational level. The ratio of energies is again in agreement with the predicted ratio for the ground-state spin of  $5/2$  for  $\text{Dy}^{161}$ . Recently we have heard that evidence has also been found for these two levels in inelastic proton scattering by Elbek at Copenhagen. The value of  $\mathfrak{S}$ , the effective moment of inertia, calculated from the energy-level spacings for the two isotopes  $\text{Dy}^{161}$  and  $\text{Dy}^{163}$ , is quite different—a rather striking result, since both have the same ground-state spin.

#### INVESTIGATION OF THE MECHANISM OF NUCLEAR REACTIONS

In recent years it has been reasonably well established that nuclear reactions other than elastic scattering proceed by a range of mechanisms lying between the two extremes of compound-nucleus formation and direct interaction. The picture of the compound-nucleus mechanism is that the incoming particle is quickly amalgamated by the target nucleus to form a highly excited compound system, the energy brought in by the incoming particle being rapidly shared by all the nucleons in the system. The compound nucleus thus formed lasts a long enough time (perhaps  $10^{-15}$  sec) to “forget” how it was formed and then decays by particle (or quantum) emission into an outgoing particle (or quantum) and a residual nucleus. The residual nucleus may be left in an excited state, and decay to its ground state by further particle or quantum emission. One possible picture of the direct-interaction mechanism is that the incoming particle

makes a two-body collision with one of the surface nucleons of the nucleus, causing the nucleon to be ejected, while the incoming particle is itself then captured by the remainder of the nucleus.

The predictions of the direct-interaction reaction model differ from those of the compound-nucleus theory, especially with regard to the angular distributions of emitted particles and the angular correlations between the incoming and outgoing particles and the gamma radiation which usually comes from the residual nucleus if that is left in an excited state.

We have investigated the angular distributions of protons and the angular correlation of protons and gamma rays in the  $F^{19}(\alpha, p)Ne^{22}$  reaction in order to characterize, at least to some extent, the reaction mechanism. The bombarding energies employed (6.0 to 6.5 Mev) are in a sense putting the direct-interaction mechanism to a rather severe test, since such a mechanism is expected to apply better at higher bombarding energies. Our results, however, show *some* striking agreements with the predictions of the direct-interaction model. The best agreement in proton angular distribution, shown in figure 16, occurred for the ground-state transition at  $E_\alpha = 6.40$  Mev. By itself, the close fit of the experimental data to the predicted yield based on the direct-interaction theory,  $[j_0(QR)]^2$ ,\* may be taken as evidence for this reaction mechanism. We observed, however, that a relatively small change in bombarding energy alters the distribution rather considerably. Since the direct mechanism should not show any "resonant" behavior, this result presumably indicates a contribution to the yield from compound-nucleus processes. This effect is shown in figure 17, in which five proton angular distributions taken at closely spaced energies are presented. Of these five, only

\*  $j_0$  is the zeroth-order spherical Bessel function; its argument is  $QR$ , where  $Q = |\mathbf{K}_\alpha - \mathbf{K}_p|$ , the  $\mathbf{K}$ 's being the wave vectors of the incident alpha particle and outgoing proton, respectively, and  $R$  being the interaction distance.

those at 6.25- and 6.40-Mev bombarding energies have shapes clearly interpretable in terms of a direct interaction. The distributions at 6.00- and 6.10-Mev bombarding energies do show peaks at approximately the proper places for the direct mechanism, although their shapes differ from the shape characteristic of the direct process. This difference in shape and, even more striking, the completely

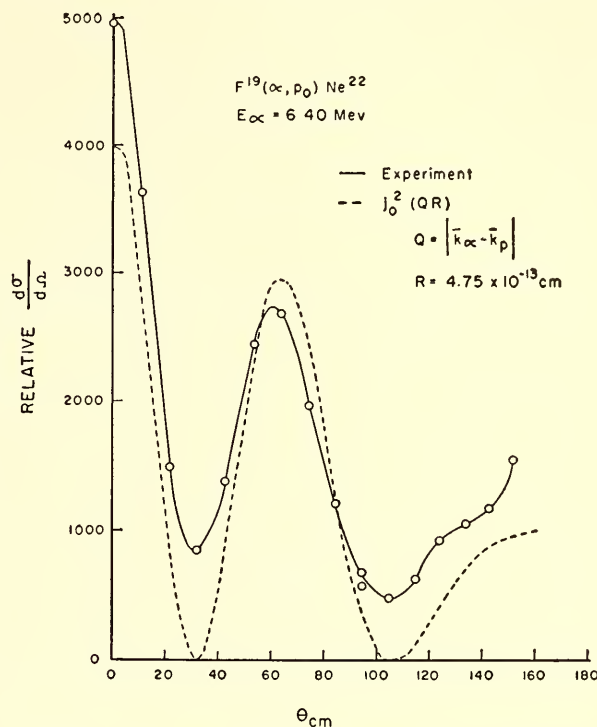


Fig. 16. Angular distribution of protons from the ground-state transition in  $F^{19}(\alpha, p)Ne^{22}$  at an alpha-particle bombarding energy of 6.40 Mev. The dashed curve shows the theoretical prediction of the direct-interaction reaction mechanism.

different nature of the distribution at 6.55-Mev bombarding energy are indications of the existence of a process additional to the direct one responsible for the result at 6.40 Mev.

The same change of distribution shape with bombarding energy was observed for the protons corresponding to the first-excited-state transition in  $F^{19}(\alpha, p)Ne^{22}$ , as will be seen in figure 18. Some of the data of the figure (e.g., the distribution at 6.10-Mev bombarding energy) can be fitted fairly well by the appropriate direct-interaction prediction for this case,  $[j_2(QR)]^2$ ,



but certainly not all the results can be so treated. The reason for this rather peculiar behavior on the part of these angular distributions as a function of bombarding energy is at present not understood in detail.

It has been pointed out by Butler, however, that at low energies contributions to the direct reaction from the interior of the nucleus would tend to alter the angular

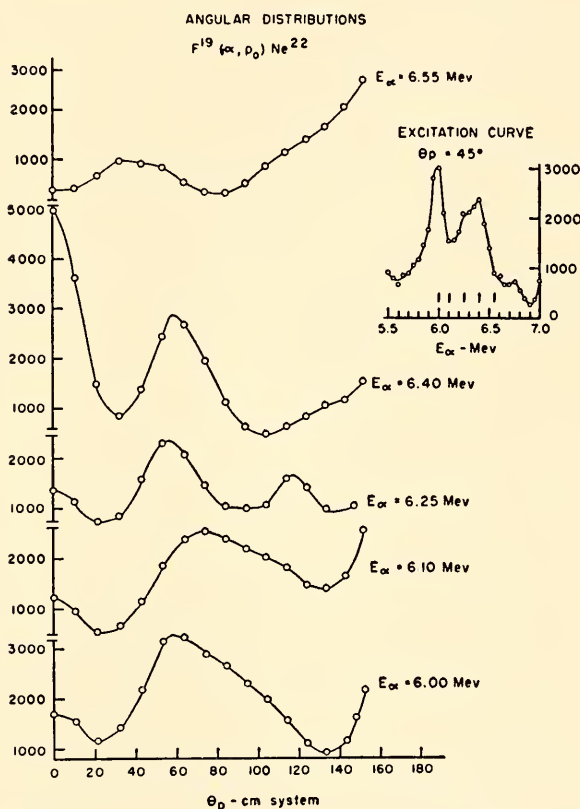


Fig. 17. Proton angular distributions in  $F^{19}(\alpha, p_0)Ne^{22}$  as a function of bombarding energy in the range of 6.00 to 6.55 Mev. The insert shows the excitation curve at  $\theta_p = 45^\circ$ ; it is not particularly representative of the total cross section.

distributions, perhaps markedly, from the expected form, and that under such conditions a measurement of the angular correlation of the gamma radiation emitted in the decay of the residual nucleus with respect to the direction of momentum transfer,  $\mathbf{Q}$ , would provide a test as to whether the reaction proceeds directly or via compound-nucleus formation. We have, for this reason, begun investigating the correlations in the reaction  $F^{19}(\alpha, p_1)Ne^{22}(\gamma)Ne^{22}$ . The theory of the correlation

for certain direct processes has been developed by Satchler. It predicts that the gamma-ray yield should be observed to be azimuthally symmetric about the direction  $\mathbf{Q} = \mathbf{K}_\alpha - \mathbf{K}_p$ , and also symmetric about a plane through the nucleus perpendicular to  $\mathbf{Q}$ . It is clear that the direction  $\mathbf{Q}$  is specified by the momenta of the alpha

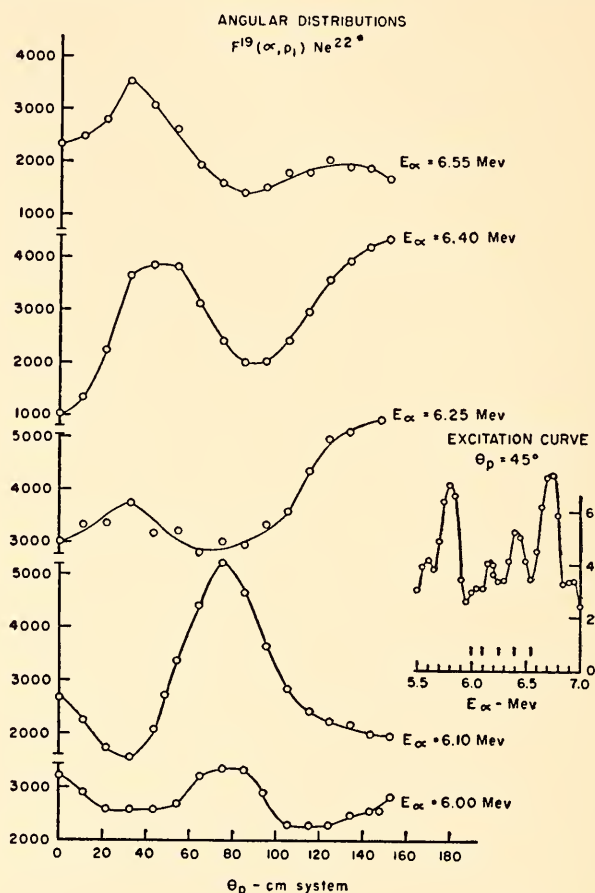


Fig. 18. Proton angular distributions in  $F^{19}(\alpha, p_1)Ne^{22}$  as a function of bombarding energy in the range 6.00 to 6.55 Mev. The insert shows the excitation curve at  $\theta_p = 45^\circ$ ; it is not particularly representative of the total cross section.

particle and proton; thus experimentally one measures the number of  $(p_1, \gamma)$  coincidences as a function of the position of the gamma-ray detector, while selectively detecting only  $p_1$  protons at a fixed position.

Our first correlation was done at  $E_\alpha = 6.40$  Mev and  $\theta_p = 40^\circ$ ,  $\phi_p = 0^\circ$  ( $\theta_p$  and  $\phi_p$  are the polar and azimuthal angles specifying the proton direction with respect to the beam direction). These angles, the bombarding energy, and the known en-

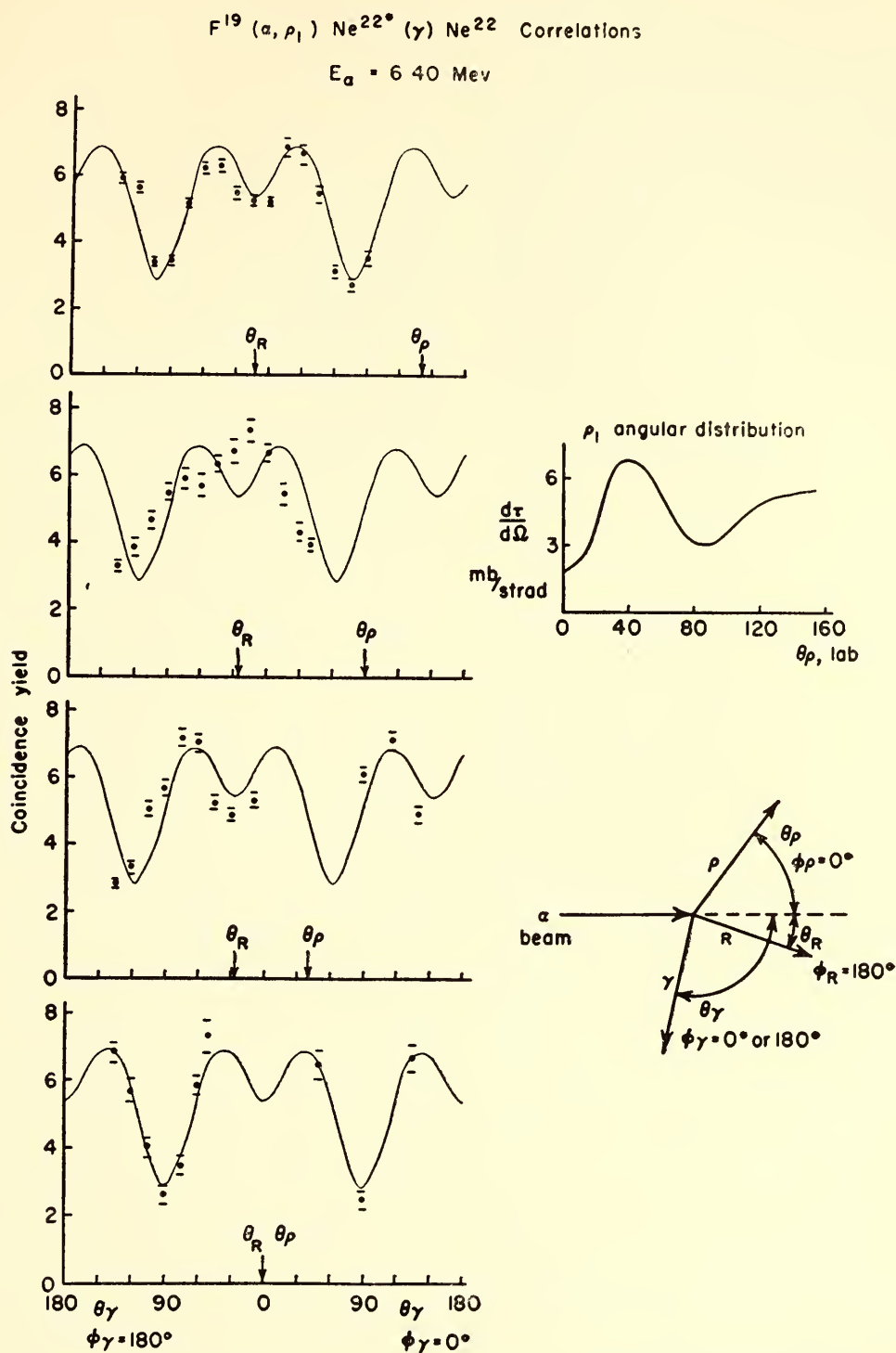


Fig. 19.  $F^{19}(\alpha, p_1)Ne^{22*}(\gamma)Ne^{22}$  angular correlations at 6.40-Mev bombarding energy, for proton detection angles  $\theta_p$  of 0°, 40°, 90°, and 140°. The solid curve in each correlation represents one particular (not unique) prediction of the direct-interaction theory; it is included to show that the results at 0°, 40°, and 140° show the symmetries with respect to  $\theta_R$  (called  $\theta_Q$  in the text) predicted by the theory, while the data at 90° do not show these symmetries. The inserts are the  $p_1$  proton angular distribution at 6.40-Mev bombarding energy and a diagram of the directions involved in the reaction.



ergy release of the reaction specify  $Q$  as having the direction  $\theta_Q=28^\circ$ ,  $\phi_Q=180^\circ$ . The gamma counter was placed at several angles  $\theta_\gamma$  for  $\phi_\gamma=0^\circ$  and  $\phi_\gamma=180^\circ$ .

The results of this and three other correlations done at 6.40 Mev for different values of  $\theta_p$  are shown in figure 19. The symmetry predictions of the Satchler theory are seen to be rather strikingly borne out by the results for  $\theta_p$  values of  $0^\circ$ ,  $40^\circ$ , and  $140^\circ$ . (The solid curve shown in each correlation has to do with the details of the theory; it is not a unique prediction, and somewhat better fits [e.g., at  $\theta_p=40^\circ$ ] can be obtained by using different values of the parameters in the theory.) The failure of the result for  $\theta_p=90^\circ$  to show the proper symmetries is equally striking. It is possible that we are observing one type of direct interaction in the forward direction and another type in the backward direction (of proton emission) while in the central region ( $\theta_p=90^\circ$ ) interference effects between the two types destroy the correlation.

In any event, the fact that a relatively simple theoretical development, based on the Born approximation, fits many of the data obtained in a quite complicated experiment is very encouraging. Further angular correlation experiments on this reaction and on similar ones, especially as a function of bombarding energy, should throw additional light on the actual processes involved in nuclear reactions.

#### BIOPHYSICS

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For ten years the aim of our studies in biophysics has been the understanding of the processes by which protein and nucleic acid are synthesized. Although this goal has been constant, the activities leading toward it have varied greatly. During

the first years our efforts were confined to observations of the synthesis of relatively small molecules. Later they shifted to studies of the metabolic pools which are the precursors of the macromolecules. It is not sufficient, however, to consider merely the gross chemical fractions of the cell, the protein, nucleic acid, lipid, and other fractions; it is equally important to distinguish whether a specific protein, for example, is located in the cell wall or in the cytoplasm. For many problems it may also be necessary to consider the organization of the cell in terms of some framework larger than a protein molecule. We have directed more attention this year to the organization of the cell.

Various approaches to the problem of cellular organization are possible. Studies of the metabolic pools show that they are sensitive to osmotic shock and must, therefore, be held in some osmotically sensitive structure. In yeast, two types of pools can be distinguished, of which one is an immediate precursor of macromolecules and the other is exchangeable with outside material. The application of high pressure causes an interconversion of the pools, presumably by alteration of cellular structure.

The cellular structure as a whole can be altered by formation of protoplasts which have weakened walls and lose the characteristic appearance of intact cells. These altered forms continue to synthesize protein and nucleic acid. A beginning has been made in the study of the "particles" of the cells and their role in the processes of synthesis. A new approach has been found in the study of quite large particles that form spontaneously from disintegrated cellular material. Through the use of amino acid analogues, the mechanism that selects amino acids for protein synthesis has been investigated by observing the "mistakes" a cell can make in protein formation. These various items, reported in detail below, have each contributed to a better understanding of the functional operation of the cell.

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AMINO ACID POOLS IN *Escherichia coli*

Further studies have been carried out on the amino acid pools of *E. coli* in order to understand the mechanisms of pool formation and maintenance. The work has been guided by the general idea that a detailed understanding of the first step in amino acid incorporation by the cell will supply a foundation for investigations of the later steps leading to macromolecule synthesis, and may supply clues to the mechanisms involved in the later steps.

Pool formation is an expression of the ability of the cell to obtain nutrients present at very low concentrations in the environment and to supply them to the synthetic machinery at high concentrations. This, perhaps, allows significant simplification of subsequent problems of macromolecular synthesis.

The principal question is whether the internally concentrated substances are free in solution within the cell or held in a more complex fashion. If the pool is simply a concentrated solution that pervades the cell, then the synthetically active structures within the cell are bathed in this solution, which is thus the "medium" in which synthesis occurs. On the other hand, the amino acids of the pool may be more closely associated with the substructures of the cell responsible for protein synthesis. They might be trapped in such substructures (as in a brush heap) or be bound to them by labile chemical bonds. In the latter case it would be highly important to know the nature of the binding sites and how intimately they are related to the synthetic activities.

The experimental work described below was aimed at distinguishing between these alternatives. Although no directly conclusive experiments were devised, the totality of the experimental evidence obtained showed that various simplified models based on these alternatives were inadequate. The elaboration of the properties of the pool obtained by these studies provides a list of critical requirements that

must be met in the formulation of any satisfactory model.

*Studies of the rate of formation, exchange, and loss from the pool.* Previous studies have shown that the pool formed by growing cells is proportional to the quantity of amino acid supplied, until the pool approaches its saturation value. A typical curve from which the pool size and rate of pool formation are estimated is shown in figure 20. Of more direct interest for the analysis of the mechanism of pool formation are the relationship of the pool size to the external concentration

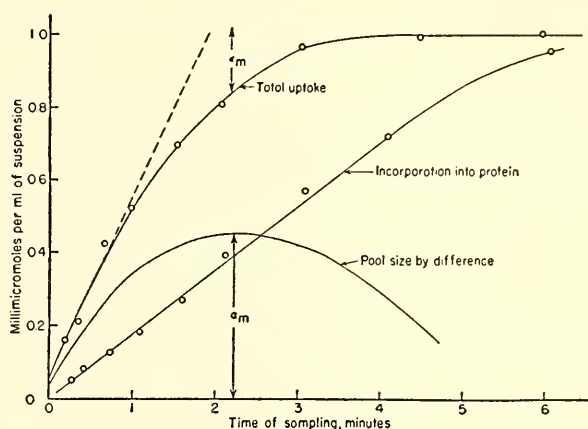


Fig. 20. Typical curve for the uptake of  $C^{14}$  proline by *E. coli* at  $25^{\circ}C$ . Initial proline concentration  $10^{-6} M$ ; cell concentration 0.52 mg wet cells/ml. The initial total rate of uptake of proline may be calculated from the slope of the dashed line. The steady-state pool size and the external concentration at the time it is achieved are determined from the values of  $\alpha_m$  and  $\epsilon_m$  shown.

present at the time a steady-state pool is achieved, and the initial rate of pool formation. For the purposes of this discussion the steady-state pool size is defined as the size of the pool at the time its rate of change is zero. A large number of experiments with  $C^{14}$  proline over the range of concentrations from  $10^{-6} M$  to  $2 \times 10^{-4} M$  have been analyzed in order to study the variation of these two parameters with external concentration. The steady-state pool size varies by a much larger factor than the initial rate of pool formation. A mechanism sufficient to explain these results will necessarily be much more com-



plicated than the "permease" mechanism proposed by Monod et al. (1956) for the concentration of galactosides in *E. coli*.

Studies have also been carried out on the rate of loss from the pool when the external amino acid is removed. When samples of cells at 25° C are caught on a filter and washed with the usual growth medium (but no glucose) for varying periods, a rapid loss of about 20 per cent of the pool is followed by a very slow loss. These experiments are complicated by the continuation of protein synthesis, and as

Exchange is observed with about the same time constant as in *A*. For experiment *C* another part of the culture was diluted by a factor of 30 and correspondingly large samples were taken. There is loss from the pool at a slower rate than the exchange process shown in *B*. Experiment *D* is similar to *C* but in place of the dilution the samples were washed on the filter for varying lengths of time with the usual growth medium (with glucose omitted). The time constant for *D* is similar to that for *C*.

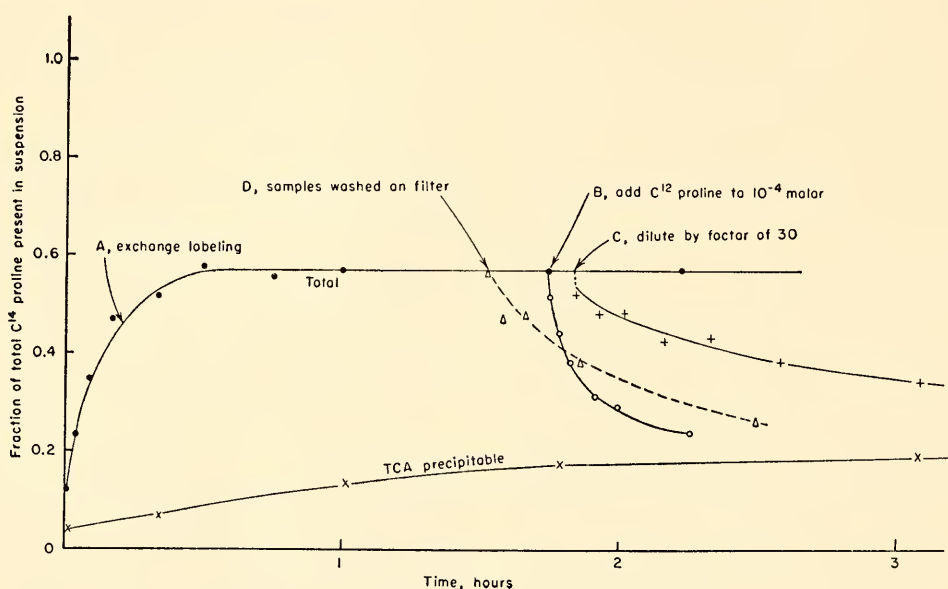


Fig. 21. Study of rate of exchange and loss of  $C^{14}$  proline pool in *E. coli* at 0° C. Cell concentration 1.0 mg wet cells/ml. Suspension incubated at 25° C for 5 minutes after addition of  $10^{-5}$  M  $C^{12}$  proline and quickly chilled to 0° C. Cells then centrifuged and resuspended in unsupplemented medium at 0° C. After 1 hour,  $C^{14}$  proline was added for experiment *A*. In experiment *C*, a part is diluted by a factor of 30 with supplemented medium. For curve *D* samples were washed on the filter for the times indicated with unsupplemented medium.

a result good quantitative measurements of the loss at 25° have not yet been obtained. The rate of loss after removal of the external amino acid, however, is very much slower than the initial rate of uptake when the amino acid is added.

Similar studies have been carried out at 0° C where protein synthesis is suppressed and more accurate rate measurements can be made. Figure 21 shows the results of an experiment performed at 0° C in which the pool was labeled by exchange *A*. The culture was then divided, and to one part, *B*, carrier  $C^{12}$  proline ( $10^{-4}$  M) was added.

In another experiment (fig. 22) the rate of pool formation was measured at 0° C. It will be observed that the rate of formation of pool (time constant 4 hours) is considerably slower than the rate of loss (time constant <1 hour, fig. 21, *D*).

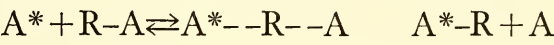
These results at 0° C contrast sharply with those at 25° C, where pool formation is a much faster process than loss. In spite of this inversion of the relative rates of formation and loss, the pool sizes at 25° and 0° C are the same within a factor of 2. It appears that the equilibrium pool size must be determined by other

parameters in addition to the relative rates of formation and loss, as measured above.

The results suggest that the existence of a pool suppresses the pool-formation step, and that the existence of an appropriate external concentration suppresses the loss step. Appropriate temperature coefficients of the four processes (pool formation and its suppression, pool loss and suppression) might account for the observations.

Results reported in last year's annual report have demonstrated that exchange between pool and external amino acid is an important process during pool formation at 25° C with an energy source pres-

ent either through reactions that are an essential part of the over-all mechanism of pool formation or through reactions that play no real part in that process. In connection with the latter case, it should be noted that a reaction of the type



where R-A is some complex containing A, would be observable in an exchange study but would not necessarily be observable in the process of pool formation, since the reaction causes no net change in the amount of the complex.

TABLE 10. Approximate Rates of Formation and Exchange

The values are expressed in millimicromoles of proline per minute per milligram wet cells at an external concentration of  $3.5 \times 10^{-6}$  M C<sup>14</sup> proline. No glucose was present in the exchange experiments.

Tem- perature, ° C	Rate of	
	Pool Formation	Exchange
0 .....	0.0074	0.18
25 .....	2.0	0.63

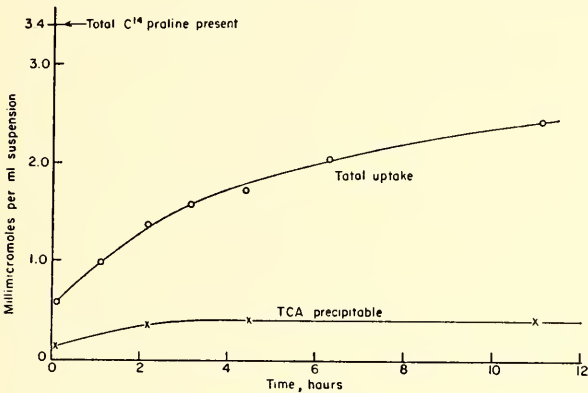


Fig. 22. Proline pool formation at 0° C, 1.1 mg wet cells/ml suspension;  $3.4 \times 10^{-6}$  M C<sup>14</sup> proline. Exponentially growing cells chilled to 0° C for 45 minutes before C<sup>14</sup> proline was added.

ent. Exchange was found to continue in the absence of an energy source, or at 0° C, conditions that strongly suppressed pool formation. Current studies show that the exchange rate has a relatively small temperature coefficient whereas the rate of pool formation has a very large one (at least, at low temperature, table 10).

Table 10 lists approximate values for the initial rates of pool formation and exchange at 25° and 0° C.

Any satisfactory model of the amino acid pool must certainly allow for the occurrence of exchange in the absence of an energy source and for the strikingly different temperature dependence of the exchange process and the process of pool formation. The exchange process may oc-

*Miscellaneous properties of the amino acid pool.* As mentioned in the last annual report, cells suspended in high-osmotic-strength media have higher saturation values of pool size. For example, when proline (0.07 mg/ml) is supplied to cells suspended in 1.3 osmolal medium (four times the strength of the usual growth medium), a pool of about 1000 μM/g dry cells is observed. When 20 mg/ml of casein hydrolysate is supplied in the same medium (1.4 osmolal), however, the total pool for *all* the amino acids is only about 1000 μM/g dry cells. Thus, whereas small amino acid pools are unaffected by the presence of other amino acids (except for certain interactions of similar amino acids, such as valine, leucine, and isoleucine) the large amino acid pools interact strongly, and are clearly not held by mechanisms specific for each amino acid.



In order to gain insight into the nature of pool binding, experiments were carried out to determine the temperature change in a thick suspension of cells when a large amino acid pool was released by osmotic shock. The results indicated that very little heat was released or absorbed during the process. The experimental limit was about 2 kcal/mole of amino acid pool.

Some exploratory studies have been made on the reaction of hydroxylamine with the pool amino acids of *E. coli*. Hydroxylamine reacts rapidly with acyl phosphates, anhydrides, and halides to form hydroxamic acids; the corresponding carboxylates, amides, and peptides react very slowly. If the pool amino acids were present as activated forms such as acyl phosphates, a fairly efficient conversion to the corresponding amino hydroxamic acids would be expected in the presence of high concentrations of hydroxylamine. Experiments to test this possibility show very small yields of amino hydroxamic acids. In the two cases which were tested with great sensitivity, identifiable quantities of leucine and tyrosine hydroxamic acids have been observed. These quantities, however, correspond to a very small fraction of the total pool of these amino acids.

In "Mg-deficient" media the ability to form amino acid pools in *E. coli* was markedly reduced, as was the rate of incorporation into the TCA-precipitable fraction. Reductions of as much as a factor of 5 in pool size have been observed, but the results are quite variable, presumably as a result of uncontrolled traces of Mg present in the "Mg-deficient" media.

When *E. coli* cells are exposed for a few minutes to a hydrostatic pressure of 20,000 psi, a large part of the amino acid pool is released to the medium. Even though growth of the cells is inhibited for an hour or so after the pressure is removed, most of the released pool is quickly reincorporated. The sensitivity of the pool to hydrostatic pressure presumably results from the distortion of the structures holding the amino acid pool, and is probably related

to the sensitivity of the pool to osmotic shock.

*Osmotic effects.* Further studies have been carried out on the effect of osmotic strength on the amino acid pool in the hope of obtaining insight into the mechanisms that hold the pool.

The pool size at low amino acid concentration is independent of the osmotic strength of the medium. When a sudden drop in osmotic strength occurs, however, the pool is partly removed and after a short time recovers to its previous value if the amino acid concentration remains constant. These results were reported in last year's annual report without interpretation. In combination with later evidence, they lead to the following picture of the process of osmotic shock.

The cell is initially in osmotic equilibrium with its normal medium. When the osmotic strength is suddenly reduced there is first a flow of water into the osmotically sensitive structures along with a slow loss of solute from the cell. The consequent stretching of the structures due to the internal pressure increases the permeability to the solute, allowing a faster rate of loss of solute molecules. These two processes together finally lead to a new osmotic equilibrium. The dynamics of this process are such that the cell passes through a transient state in which the structures are distended. The loss of pool is associated with this loosening up. The structures responsible for the holding of the pool may be either the cell wall or membrane or internal constituents normally constrained by the cell wall.

Since competing rates of flow of water and solute are involved, this picture suggests that a slow change in the osmotic strength might be less effective than a rapid shock. Observation bears out the suggestion. In an experiment with a large proline pool a reduction in osmotic strength of a factor of 3 was made in four steps of equal concentration ratio. As a result, 70 per cent of the pool was removed. If the

same final osmotic strength was achieved in a single step, 90 per cent was removed. Further, when the same final strength was achieved through a concentration change, graded continuously over several minutes, only 50 per cent of the pool was removed.

Another implication of this description of the process of osmotic shock is that molecules which do not ordinarily enter the osmotically sensitive structures might be able to diffuse in during the transient period when the permeability is increased. In our laboratory jargon this process has been called “trick or treat,” since it was suggested just after Halloween and brings to mind the children’s trick of throwing in orange peels while the door is open.

This transient permeability was tested experimentally by giving a thick suspension of cells at 0° C a sudden osmotic shock in the presence of radioactive SO<sub>4</sub><sup>2-</sup> or PO<sub>4</sub><sup>3-</sup>. The suspension was then diluted without osmotic shock to remove diffusible label, and filtered. A small amount of SO<sub>4</sub><sup>2-</sup> or PO<sub>4</sub><sup>3-</sup> was taken up, corresponding to about 5 per cent of the cell volume at the external concentration of SO<sub>4</sub><sup>2-</sup> or PO<sub>4</sub><sup>3-</sup>. Various controls showed that this uptake was indeed due to the sudden downward change in osmotic strength. Upward osmotic shocks neither remove the pool nor cause the trick-or-treat phenomenon.

This phenomenon may shed some light on earlier observations of the paradoxical conditions required for efficient production of mutations with Mn<sup>++</sup>, as described in the annual report of 1951–1952 (Year Book 51). Effective production of mutations occurred when cells suspended in saline were transferred to low-osmotic-strength medium containing Mn<sup>++</sup>.

Studies have been made of the osmotic effectiveness of various solutes for protection of a preformed pool or its removal by osmotic shock. The experiments were performed by collecting on a filter cells containing a C<sup>14</sup> proline pool, and briefly washing (5 seconds) with the following types of solutions: (A) the solute at the

same osmotic strength as the growth medium (0.37 osmolal) dissolved in growth medium; (B) the solute at this osmotic strength dissolved in water; and (C) solution as in A, followed by a wash with the usual growth medium. Table 11 summarizes the results.

TABLE 11. Osmotic Effects of Various Solutes on *E. coli* Proline Pool

Solutes	Percentage of Pool Removed		
	A, upshock	B, protec- tion	C, upshock and down- shock
Butanol . . . . .	77	100	77
Diethylene glycol . .	30	70	95
Ethyl acetoacetate . .	50	90	50
Methanol . . . . .	20	100	20
Ethanol . . . . .	20	100	20
Propanol . . . . .	20	100	20
Acetone . . . . .	10	100	15
Propionamide . . . . .	10	100	20
Succinimide . . . . .	20	100	20
Acetamide . . . . .	20	100	50
Dioxan . . . . .	5	95	40
Glycerol . . . . .	5	99	70
Urea . . . . .	5	97	63
NaCl . . . . .	0	38	55
NaAC . . . . .	3	60	50
Diethylamine HCl . .	6	35	60
Tris HCl . . . . .	8	35	55
Glycine . . . . .	1	16	47
Alanine . . . . .	3	12	50
Valine . . . . .	5	12	60
Proline . . . . .	5	30	60
Glucosamine HCl . . .	0	5	50
Xylose . . . . .	0	10	52
Glucose . . . . .	0	10	52
Galactose . . . . .	0	10	45
Sucrose . . . . .	0	5	70

- A. Washed on filter with solute at 0.37 osmolal in growth medium.
- B. Washed on filter with solute at 0.37 osmolal in H<sub>2</sub>O.
- C. As in A followed by wash with growth medium.

rizes the results. Since, in general, upward osmotic shock has no effect on the pool, A simply measures possible chemical or destructive effects on the cells. Compounds such as butanol remove most of the pool. Washes of type B measure the effectiveness



of the solute as an osmotic protector for short periods of time. It will be seen that, in general, high-molecular-weight compounds are most effective, although the zwitterion glycine (M.W. 75) is an effective protector but glycerol (M.W. 92) is not. It is presumed that solutes which do not act as protectors are able to enter the osmotically sensitive structures rapidly. The resulting excess water activity outside causes water to flow in until the distension allows internal osmotic constituents to leak out, re-establishing equilibrium.

Washes of type *C* measure the effect of a sudden doubling of the osmotic strength followed rapidly by a return to the usual medium. Compounds like acetone which enter the cell rapidly without causing damage (as shown by the figures in columns *A* and *B*) do not cause significant removal by downward osmotic shock. On the other hand, compounds like urea and glycerol, which are not effective protectors, are capable of removing the pool by osmotic shock.

A sudden increase of the osmotic strength of the medium causes a quite different effect from a sudden decrease. When a growing cell suspension is suddenly mixed with an equal volume of medium containing 1 *M* sucrose, synthesis of nucleic acid and protein stops. After a period of roughly 8 minutes, the synthetic activities resume; during this period the metabolic pool materials continue to be incorporated. Similar effects are observed with glucose and sodium chloride, but proline causes no major effect.

If the cells are washed with Tris medium just after sucrose is added, there is no loss of  $P^{32}$ -labeled pool materials. At the end of 8 minutes a Tris wash removes half the pool. It appears that the sucrose penetrates slowly into some structure of the cell associated with holding the  $P^{32}$ -labeled pool. Before the sucrose has penetrated, the structure is perhaps dehydrated, causing the cessation of synthesis. After the sucrose has penetrated, the struc-

ture can be osmotically shocked by washing the cells with Tris. It is striking that the period for this action is quite different from the period required for sucrose to penetrate the cell wall of lysozyme-treated cells in the procedure for protoplast formation. It may also be significant that glucose has the same action, whereas it is less effective in the protoplast procedure (see below).

An observation of considerable interest from the point of view both of the osmotic properties of the cell and of the mechanism of pool formation is that different types of pools appear to have different sensitivity to osmotic shock. Both amino acid pools and  $P^{32}$ -labeled pools are completely removed by a quick water wash at room temperature; at 0° C, however, while the amino acid pools are still completely removed, only half of the  $P^{32}$ -labeled pool is removed. Osmotic shocks (at room temperature) that remove half of the amino acid pool will remove considerably less of the  $P^{32}$ -labeled pool. It also appears that when very large amino acid pools, formed at high osmotic strength with casein hydrolysate, are partially removed by osmotic shock, the amino acid distribution is considerably altered.

These observations indicate that various pool materials are organized in different ways within the cell, and perhaps are associated with different substructures.

Some studies have been made, by means of freezing-point measurements, of the release of the total osmotically active material of the cell by osmotic shock. The results are broadly similar to those of studies of amino acid pools. Water washes remove the total osmotically active constituents almost completely. Boiling of the cells after water washing releases only traces of additional material effective in depressing the freezing point of water. When the osmotic shock is performed in small steps, the release of the total osmotically active material is similar to the release of amino acid pools, though perhaps a somewhat greater per-

centage is released for the same shock. The total released from the cell indicates that, if this material is osmotically active when present in the cell, the osmotic pressure within the cell is slightly greater than that of the medium and is dependent on the osmotic strength of the medium. If this material were concentrated in regions smaller than the whole cell, the osmotic pressure would be proportionately higher.

Unusual behavior is exhibited by a thick water suspension of water-washed cells. If such a suspension is cooled somewhat below  $0^{\circ}\text{C}$  (in a vigorously stirred freezing-point cell), and an ice crystal is added to seed the crystallization, the temperature rises initially as in a typical freezing-point experiment. The temperature rises higher than  $0^{\circ}\text{C}$ , however, and continues to rise slowly and steadily as heat is extracted. When the temperature reaches about  $+0.7^{\circ}\text{C}$ , stirring of the mixture becomes difficult. If the system is now removed from the cooling bath and allowed to stand (with stirring) in the air, the temperature falls. The fall can be made more precipitous by immersing in hot water. If the system is returned to the cold bath ( $-2^{\circ}\text{C}$ ), the temperature rises. This anomalous behavior, an apparent negative heat capacity, is reversible provided that the temperature is kept in the range of 0 to  $+0.7^{\circ}\text{C}$ . The cause of this apparent negative heat capacity has not yet been determined.

*Summary of the properties of the metabolic pool in E. coli.* The properties of the pool that must be incorporated in any model may be summarized as follows.

1. Energy is required for formation of the pool but not for maintenance of a preformed pool.

2. Pools are formed very slowly at  $0^{\circ}\text{C}$ , and preformed pools are maintained.

3. Exchange occurs independently of any energy source. The temperature coefficient of the exchange process is small; that for formation is very large.

4. Small pools are not influenced by the presence of other amino acids, but large pools are suppressed.

5. When the external amino acid is removed, the pool is lost at a much slower rate than that at which it was initially formed.

6. The initial rate of pool formation and the pool size do not have the same dependence on amino acid concentration.

7. Pools are removed by osmotic shock, but not all pool compounds are removed equally.

8. The saturation value of the pool is roughly proportional to the osmotic strength of the medium.

As yet no model incorporating all the features necessary to provide for the eight properties listed above has been worked out in sufficiently critical detail to be tested against the available data.

#### AMINO ACID POOLS IN YEAST

Kinetic studies have shown that in *Candida utilis* pool formation is a preliminary and necessary step in macromolecule formation. For nucleic acid synthesis two chemically distinct and functionally different purine pools are known. The first, a concentrating pool, accumulates nucleic acid bases within the cell at levels exceeding their external concentrations. This pool is evident only when the synthetic medium is supplemented with bases. The pool size is variable and dependent upon external concentration. Once concentrated, these bases may provide material for the second, a nucleotide pool, which is always present and remains constant in size during exponential growth. Here conversion of one nucleotide to another occurs, furnishing the appropriate molecules for nucleic acid synthesis.

Evidence has accumulated indicating the existence of two functionally distinct amino acid pools (in yeast) analogous to these pools of purine compounds.

*Kinetic studies of pool formation from exogenous amino acids.* It has been shown previously that during exponential growth in media containing fructose as the sole carbon source 13 per cent of the cellular carbon is contained in an amino acid pool.



It is now known that the total amount of amino acid pooled in the cell can be increased by the addition of amino acids to the growth medium.

Exogenous amino acids are quickly incorporated by exponentially growing cells. Figure 23 shows the time course of incorporation of trace quantities of C<sup>14</sup> glutamic acid. The tracer “pulse” appears first in the cold trichloroacetic acid (TCA)-soluble fraction, and, as the small quantity of exogenous amino acid becomes exhausted, the transfer of pool radiocarbon (cold-TCA-soluble fraction) to the protein fraction is observed. These results resemble

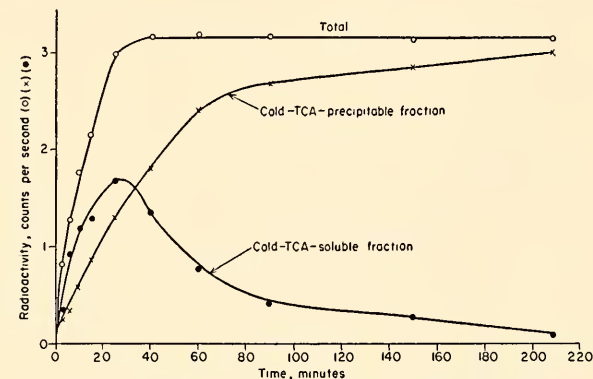


Fig. 23. Time course of incorporation of tracer quantities of C<sup>14</sup> glutamic acid. At time=0, the exogenous concentration was 0.002 mg glutamic acid/ml medium.

those obtained in the investigation of the kinetics of incorporation of exogenous purines, and demonstrate that the incorporated amino acid is contained in a metabolic pool which may supply amino acid carbon for protein synthesis. Similar results have been obtained with a variety of C<sup>14</sup>-labeled amino acids supplied to *C. utilis* growing exponentially in C medium.

When concentrations of exogenous amino acids are higher, the quantity of amino acid contained in the cold-TCA-soluble fraction increases and exceeds the external concentration. Table 12 shows the accumulation of C<sup>14</sup> threonine in the cold-TCA-soluble fraction with increasing concentrations of exogenous threonine. For comparison, table 13 shows the steady-state distribution of pool amino acid and protein amino acid found in cells grown in C

medium when fructose was the sole carbon source. These data demonstrate that the size of the pool concentrated from exoge-

TABLE 12. Distribution of C<sup>14</sup> Threonine Carbon among Pool and Protein Amino Acids\*

Concentration of Exogenous C <sup>14</sup> Threonine, μM/ml medium	C <sup>14</sup> Threonine, μM/g dry weight cells †	
	Cold-TCA-Soluble Fraction	Cold-TCA-Precipitable Fraction
50.0.....	442	520
8.4.....	354	446
6.7.....	268	328
5.0.....	190	272
3.4.....	104	149
1.7.....	32	82
0.8.....	9	41

\* Cells grown from light inoculum to about 2.8 mg wet weight of cells per ml medium.  
† Calculated on the basis that all the radio-carbon incorporated remained C<sup>14</sup> threonine.

TABLE 13. Steady-State Distribution of Radio-carbon among Pool and Protein Amino Acids\*

Component	Pool Quantity of Compound, μM/g dry	Protein Quantity of Compound, μM/g dry
Isoleucine-leucine .....	117	785
Lysine .....	24	625
Glutamic acid .....	290	640
Aspartic acid .....	9	762
Valine .....	65	512
Alanine .....	240	695
Threonine .....	8	455
Serine .....	8	600
Proline .....	7	287
Arginine .....	63	210
Glycine .....	108	488
Per cent accounted for..	87	85

\* Data obtained from cells growing exponentially in C medium containing C<sup>14</sup> fructose.

nous amino acids depends on the external concentration and may greatly exceed the pool formed endogenously from fructose. Chromatographic examination of hydrolysates of the cold-TCA-soluble and

precipitable fractions of cells grown at the highest concentration of exogenous threonine (50  $\mu\text{M}/\text{ml}$  medium) showed that more than 90 per cent of radiocarbon in the TCA-soluble fraction was contained in threonine, with the remainder in isoleucine. In the protein fraction, however, the ratio of radioactivity in these two amino acids was about one-to-one.

*Amino acid pool characteristics.* The size of pool formed from fructose carbon is constant. Furthermore, the addition of high concentrations of exogenous amino acid produces no immediate dilution or exchange with the *existing* pool. The transfer to protein of *these pool amino acids* is uninterrupted by the addition of the amino acid supplements. On the other hand, the exogenous amino acids are rapidly incorporated by the cell (fig. 23) and accumulated to levels exceeding their external concentration. Thus, there are two pools of amino acids, one formed from fructose, the other from exogenous amino acids; for convenience, they will be called the *internal* and *expandable* pools of amino acids, respectively.

The amino acids of the internal pool are not lost to the medium during exponential growth, nor are they exchanged with exogenous amino acids. In the expandable pool, however, the situation is quite different, as can be seen from the results of experiments on cells grown in C medium containing fructose and supplemented by a high concentration of  $\text{C}^{14}$ -arginine (1.0 mg/ml medium). The kinetics of incorporation of radiocarbon into pool and protein, followed during this labeling growth period, showed that approximately two-thirds of the protein arginine was being derived from fructose carbon and one-third from the amino acid supplement. After  $3\frac{1}{2}$  hours of growth in this medium the cells were harvested and washed twice with C medium, and an aliquot was transferred to C medium containing nonradioactive arginine (1.0 mg/ml, culture A). Since the quantity of amino acid incorporated during growth in the labeled me-

dium was quite small in comparison with the total quantity present, the cells were exposed to an essentially constant environment of exogenous arginine. Another aliquot was added to C medium containing no arginine (culture B). At the time of transfer, 57 per cent of the incorporated radiocarbon was contained in the cold-TCA-soluble fraction. Optical-density measurements indicated that growth proceeded after the transfer with no delay in the unsupplemented culture (B) and with

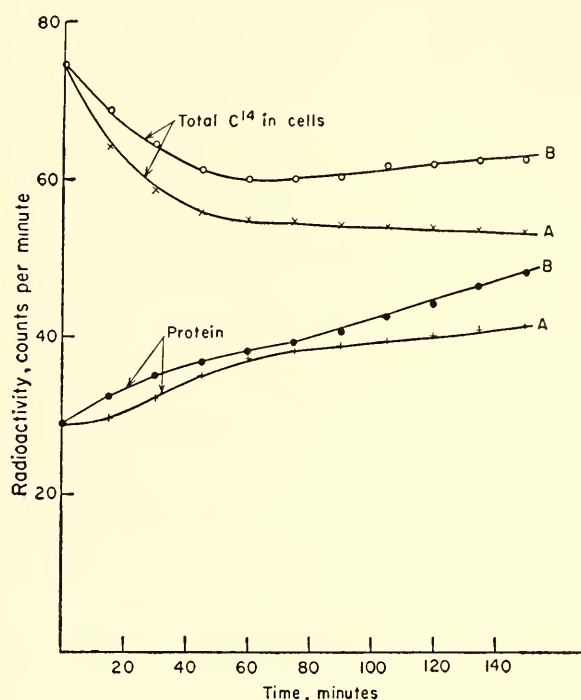


Fig. 24. Upper curves=loss of radioarginine from cells after transfer to nonradioactive C medium containing 1.0 mg  $\text{C}^{12}$  arginine/ml (A) or unsupplemented C medium (B). Lower curves=incorporation of radioarginine carbon into protein fraction of *C. utilis* after transfer of labeled cells to nonradioactive C medium (A and B as above).

only a slight delay (10 min) in the other (A).

Both groups of cells lose radiocarbon to the medium, as is shown in figure 24, but the loss in B (no supplement) is less rapid than in A. In B, reincorporation of the undiluted radioactivity becomes evident in about an hour. These results may be interpreted as follows. Where no supplement was added, the expandable pool decreases for two reasons: there is a continuous flow



into the internal pool and thence into protein, and there is an approach to a new equilibrium with the medium. When the expandable pool has reached a small enough value through these two processes, and the external concentration has reached a large enough value, incorporation of radiocarbon into protein again increases. In experiment *A*, the loss of expandable-pool radiocarbon is due to transfer through the internal pool into protein, and to exchange with the nonradioactive arginine in the medium. This exchange process is faster than the loss of pool material to the medium in *B*. These results are similar to those obtained with *E. coli* discussed earlier in this report. The continuous and large dilution in experiment *A* results in a steadily decreasing specific radioactivity of both pools. Consequently, there is a steadily decreasing rate of radiocarbon incorporation into the protein. The protein-incorporation curve can be quantitatively predicted on the assumption that the cell processes as measured in the 3½-hour pre-labeling continued (except for the slight lag), and the only change in circumstances was the introduction of the exchange of radioactivity between the pool and medium.

The rate of incorporation of radiocarbon into protein in the case of the unsupplemented medium (*B*) is not immediately changed by the transfer, but it falls as radiocarbon from the expandable pool is lost to the medium. Apparently the proportion of the flow of arginine carbon through the internal pool, which is derived from the expandable pool (as contrasted to that derived from fructose), is a function of the size of the expandable pool. As the flow from the expandable pool decreases, the specific radioactivity of the internal pool also decreases, and there is a consequent reduction in the rate of incorporation of radiocarbon into protein.

In figure 25 the logarithms of the radioactivity in the cold-TCA-soluble fraction in the two experiments described above are plotted against the time after the transfer

to the nonradioactive medium. In both experiments the data can be approximated by a pair of straight lines. In *A* the early, fast component presumably represents the combined effects of exchange and transfer to protein via the internal pool, whereas the slow component reflects the decrease in specific radioactivity of the internal pool. Since the internal pool is constant in size and undergoes no exchange, the rate of dilution of its specific radioactivity is limited by the rate of protein synthesis. In *B*, the slower component again reflects

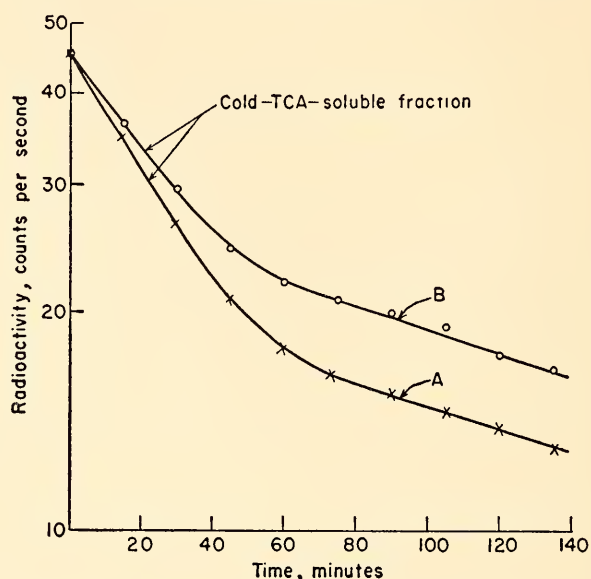


Fig. 25. Loss of pool radioarginine after transfer of labeled cells to nonradioactive C medium (*A* and *B* as in fig. 24).

the transfer of internal pool carbon to protein while the faster component includes the approach to the new equilibrium between the expandable pool and the medium.

*Effect of hydrostatic pressure.* Studies have been made of the effect of pressure treatment on the stability of the amino acid pools in yeast. Exponentially growing cells were labeled by a 2-minute immersion in C medium containing carrier-free  $C^{14}$  fructose. The cells were washed by centrifugation in nonradioactive C medium, and then resuspended in C medium without fructose. These cells contained 87 per cent of the incorporated radiocarbon in the TCA-soluble fraction.

An aliquot of these cells was compressed in a pressure cell at 30,000 psi. This pressure was rapidly applied and released 10 times over a 5-minute interval.  $C^{12}$  fructose was added to the treated culture, the cells were aerated at 30° C, and the growth and fate of the labeled pool carbon were measured. Figure 26 shows that 64 per cent of the incorporated radiocarbon was lost from the cells to the medium during the first 90 minutes of aeration. The remain-

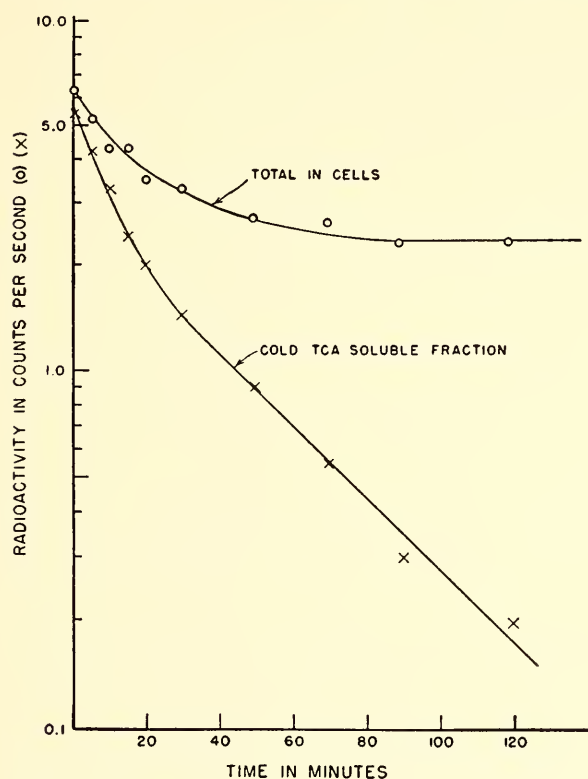


Fig. 26. Loss of radiocarbon from cells (upper curve) and metabolic pools (lower curve) after cells were subjected to a pressure of 30,000 psi.

ing pool radiocarbon was transferred to protein or nucleic acid. No increase in optical density was observed until after 100 minutes, when growth resumed, and the cells were soon growing at the optimal rate.

A control culture of these labeled cells not subjected to pressure continued to grow exponentially upon addition of fructose and lost only a few per cent of incorporated radiocarbon to the medium during the 90-minute interval.

Simple rupture of cellular membranes cannot be responsible for the release of

internal pool amino acids to the medium. No loss of the pool is observed after the pressure treatment unless fructose is added to the culture. Thus, both energy and time are required for the release of pool materials. Furthermore, microscopic examination during the course of the experiment showed no cell fragments or visible cellular alterations.

One explanation of the observed results is that the pressure treatment disrupts the pool organization and reorganization must occur before growth resumes. Evidence supporting this conclusion was obtained

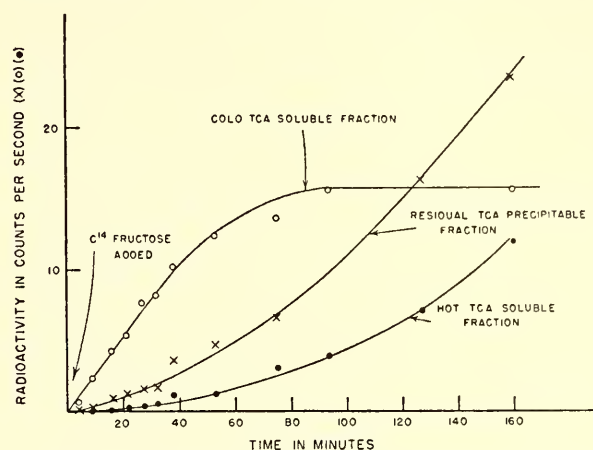


Fig. 27. Kinetics of incorporation of  $C^{14}$  fructose in *C. utilis* after cells were subjected to a pressure of 30,000 psi.

in another experiment by following the kinetics of  $C^{14}$  fructose incorporation immediately after the pressure treatment. Figure 27 shows that rapid incorporation of fructose carbon occurs. Initially this incorporation is due to pool reconstruction, since most of the incorporated radiocarbon appears in the TCA-soluble fraction. The pool appears to reach saturation in 90 minutes, at which time the quantity of radiocarbon contained in the TCA-soluble fraction corresponded to two-thirds of the normal steady-state value of the internal pool. This incorporation is approximately equal to the quantity of pool material lost from the cells (fig. 26) and therefore represents replenishment of the pool. No increase in the optical density of the cells was observed until 120 minutes after the addition of the fructose.



*The effect of osmotic shock.* The internal pool of amino acids is relatively insensitive to osmotic shock.  $C^{14}$ -fructose labeled cells lose from 3 to 8 per cent of their internal pool material when suspended for half an hour in distilled water. About the same loss is observed when these labeled cells are transferred to non-radioactive C medium. The amino acids in the expandable pool, on the other hand, behave quite differently. Cells were grown for several hours in medium containing 6  $\mu$ M  $C^{14}$  threonine/ml, then harvested and washed twice in unsupplemented medium. An aliquot was chemically fractionated, and the quantity of  $C^{14}$ -threonine carbon found in the TCA-soluble fraction

ably due to a distortion of the structures binding the pool.

*Conclusions.* Two functionally distinct amino acid pools exist in *C. utilis*. The major characteristics of these two amino acid pools are compared in table 14.

The fact that these two pools display such different characteristics and do not rapidly equilibrate with each other in the cell indicates that they are physicochemically distinct. It seems unlikely that phosphorylated forms could account for this difference, since there is insufficient pool phosphorus (300  $\mu$ M/g dry weight cells) available even for the 1000  $\mu$ M of amino acids in the internal pool. The expandable pool can be even larger than the internal

TABLE 14. Major Characteristics of Amino Acid Pools in *Candida utilis*

Characteristic	Expandable Pool	Internal Pool
Function	Concentrates exogenously supplied amino acids.	Interconverts and selects amino acids for protein incorporation.
Size	Variable and dependent upon exogenous amino acids concentration.	Fixed.
Stability	Sensitive to osmotic shock. Exchanges with exogenous amino acids.	Insensitive to osmotic shock. Not exchangeable with exogenous amino acids.

corresponded to 56  $\mu$ M/g dry weight of cells. This value is seven times the concentration found for threonine in the internal pool (see table 13). When another aliquot of these labeled cells was suspended in water the cells immediately lost 50 per cent of the total pool radiocarbon to the water.

The internal pool of amino acids in cells subjected to a pressure of 30,000 psi becomes very sensitive to osmotic shock. Cells labeled with a 5-minute immersion in medium containing  $C^{14}$  fructose were harvested and found to have 80 per cent of the incorporated carbon in the TCA-soluble fraction. An aliquot of these cells after the pressure treatment lost 31 per cent of the internal pool carbon when suspended in distilled water. A control suspension (no pressure) lost 6 per cent to the water wash. The sensitivity of the internal pool to hydrostatic pressure is prob-

pool, but no additional incorporation of  $P^{32}$  occurs when large quantities of exogenous amino acids are rapidly accumulated in the expandable pool. Thus, neither the internal nor the expandable pool can be explained on the basis of phosphorylation of the amino acids. Some correlation between phosphorus turnover and amino acid accumulation would be expected if phosphorus were associated with these amino acids.

It has been suggested that the internal pool is complexed with macromolecular components of the cell. The only class of substances present in sufficient quantity to accommodate the internal pool amino acids are the cellular proteins. A one-to-one complex between protein amino acids and pool amino acids would require that 25 per cent of the protein be so involved.

From the known molar distribution of internal pool and nucleic acid carbon there

would be 3.3 times too many amino acids for a one-to-one correlation between pool amino acid and nucleotide residues. A mixture of protein and nucleic acid could, of course, serve as a complexing system.

It seems likely that macromolecules, most probably the proteins, are the sites of association for the internal pool amino acids. These amino acids are on the main line of protein synthesis, and are more tightly bound than those in the expandable pool. The internal pool is more closely linked to the mechanisms of final protein incorporation, for it is here that amino acid interconversions and selection for protein incorporation occur.

In the expandable pool the amino acids accumulated do not interconvert, as can be seen from the data of table 12. The threonine concentrated by the cells grown on the highest exogenous threonine concentration remained, for the most part, as pool threonine, but a small portion was converted to pool isoleucine. This isoleucine and a small quantity of the total pool threonine are probably components of the internal amino acid pool. The demonstration in this experiment that protein threonine and isoleucine both become about equally radioactive even though there is a large disparity in their pool concentrations reinforces this conclusion. Figure 28 is a schematic diagram of the flow of carbon from exogenous amino acids and from fructose, via the metabolic pools, to proteins as interpreted from these studies.

These kinetic investigations describing the flow of exogenous carbon through metabolic pools and hence into protein provide a more definite picture of some of the preliminary steps in protein formation. In addition, they reveal that the amino acid composition of the cell may vary, reflecting the kind and quantity of exogenous molecules. This altered composition, in turn, affects the endogenous flow of fructose carbon which alone, in the absence of other exogenous organic molecules, is a satisfactory carbon source. Such studies demonstrate the great capacity of

some living cells to utilize ever-changing environments economically.

#### PROTOPLASTS

*The effects of lysozyme on the structure and function of E. coli.* In order to study the macromolecular composition of the bacterial cell by physicochemical methods, such as ion exchange and ultracentrifugation, it is necessary to disrupt the cell and release its contents. The use of the enzyme lysozyme, together with osmotic shock, has proved an efficient way of breaking

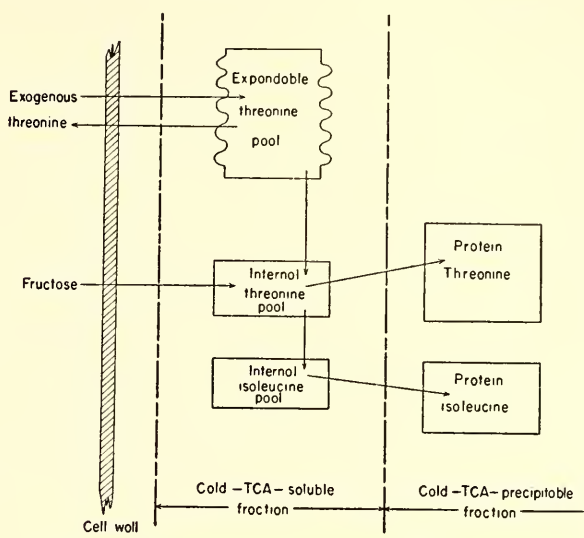


Fig. 28. Flow of threonine carbon through metabolic pools in the synthesis of protein.

*E. coli.* The conditions for lysis and physical studies of the lysates are described below. Lysozyme and osmotic shock may also be used to inflict controlled damage upon *E. coli* and thus provide altered cells, spherical forms which are usually called "protoplasts." Study of these forms should prove helpful in evaluating what structures of the cell must remain intact in order for macromolecule synthesis to occur at normal rates. Metabolic studies on the spherical forms are described in the following paragraphs.

*Lysis due to lysozyme treatment and osmotic shock.* Cultures of *E. coli* growing or resting in various media (C medium, C medium diluted with 9 volumes of water, or C medium containing 18 per cent sucrose) show no response to added lyso-



zyme (100  $\mu\text{g/ml}$ ). They neither lyse, show an impaired growth rate ( $15^\circ$ ,  $23^\circ$ , or  $37^\circ$  C), nor exhibit morphological change visible in the phase-contrast or dark-field microscopes. When such cultures are harvested, washed free of excess lysozyme by centrifugation, suspended in 0.5 M sucrose, and suddenly diluted with 10 volumes of distilled water, the bacteria continue to maintain their normal rodlike structure and upon subculture grow as well as the corresponding control (lysozyme-omitted) cultures. It appears that under these conditions lysozyme does not seriously damage *E. coli*. If, however, lysozyme is injected into a suspension of cells in 0.5 M sucrose a few seconds before sudden dilution with 10 volumes of water, nearly complete lysis occurs and a highly viscous solution containing numerous free cell walls results. An electron micrograph of several of these cells walls is shown in figure 29, plate 3. Ultracentrifugal analysis of the viscous solution shows a pattern of sedimenting components typical of those observed in cell juices of *E. coli*. A sedimentation diagram is shown in figure 30, plate 4.

The influence of molecular size of the solute particle on the ability of *E. coli* to lyse was tested by suspending cells in various solutions isotonic with 0.5 M sucrose, adding lysozyme, and diluting the suspensions suddenly with 10 volumes of water. The degree of lysis was found to increase with increasing molecular weight of the solute particle. Thus, inorganic salts (NaCl,  $\text{Na}_2\text{SO}_4$ , C medium salts) and low-molecular-weight organic compounds (urea, glycerol, Tris, sodium succinate, sodium glutamate, casamino acids) caused no change in the suspension of cells. With glycine, however, lysis occurred slowly. Xylose, sorbitol, mannitol, inositol, and glucose did not allow lysis, but the marked streaming birefringence of the suspensions was much reduced, and observation of the bacteria in the dark-field microscope revealed that 50 to 100 per cent of the cells had assumed a spherical shape. Lactose, sucrose, maltose, and raffinose allowed

complete and rapid lysis. On the other hand, when the osmotic pressure of a lysozyme-sucrose suspension of cells was slowly lowered by the dropwise addition of distilled water, lysis failed to occur. Such "decompressed" cells were morphologically and metabolically indistinguishable from untreated bacteria.

From these results it was evident that the presence of lysozyme and a *considerable osmotic shock* were necessary to cause lysis. Since osmotic shock results from a relatively high intracellular concentration of solute, the opportunity was afforded to test the rate at which various solutes entered and left the cell. When *E. coli* were suspended in 0.5 M sucrose-lysozyme solution for varying lengths of time and suddenly diluted, complete lysis occurred only after 30 to 60 seconds' equilibration of the cells with the suspending medium. Alternatively, when bacteria that had been equilibrated with a 0.5 M sucrose solution were first suddenly diluted and then lysozyme was added after increasing intervals, lysis occurred only in those cells that had less than 1 minute's exposure to the dilute medium. Thus, enough sucrose to cause osmotic lysis can enter or leave *E. coli* cells in about a minute.

*Amino acid utilization by E. coli protoplasts.* *E. coli* protoplasts may be efficiently prepared from exponentially growing cultures by suspending the cells in 0.5 M sucrose in C or Tris medium, adding lysozyme (100  $\mu\text{g/ml}$ ), and suddenly diluting the suspension with an equal volume of water. In about 20 minutes at  $25^\circ$  C (10 min at  $37^\circ$  C) the suspension consists of 85 to 95 per cent protoplasts. Under these conditions protoplast formation requires considerable time and is a temperature-sensitive process. The walls of these forms enclose volumes from 2 to 50 times those of the rodlike forms. The protoplasmic body within the wall may occupy the entire volume of the protoplast, may frequently be observed in a doubled condition within a single outer wall, or may adhere to one side of the wall, yielding a saucer-shaped inner structure. Since such proto-

plasts are morphologically altered *E. coli*, it was pertinent to inquire whether the structural changes would influence the maintenance and formation of amino acid pools and the synthesis of protein.

When growing *E. coli* were allowed to metabolize  $C^{14}$  proline in the presence or absence of lysozyme, no differences in the utilization of the amino acid could be detected. Equal  $C^{14}$ -proline pools were formed, and protein synthesis continued, as shown in figure 31. Lysozyme-treated

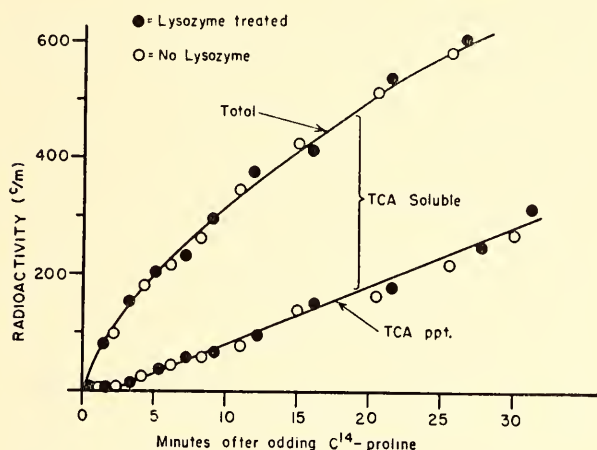


Fig. 31.  $C^{14}$ -proline utilization by cells growing in the presence of 100  $\mu\text{g/ml}$  lysozyme.

cultures grow indefinitely at the rate characteristic for *E. coli*. Thus, lysozyme treatment in itself had no apparent effect upon amino acid utilization or growth of *E. coli*.

Cells that had previously formed a  $C^{14}$ -proline pool and synthesized protein continued to maintain a large fraction of the original pool, increased the size of the pool, and continued to synthesize protein at a high rate even though they were undergoing the morphological change, rod-to-sphere, which followed lysozyme-sucrose osmotic-shock treatment. These findings are illustrated in figure 32.

The results of a more stringent test of the capacity of *E. coli* protoplasts to form an amino acid pool and to synthesize protein are shown in figure 33. For this experiment a protoplast suspension containing 96 per cent spherical forms was prepared and allowed to utilize  $C^{14}$  proline. It is evident that an amino acid pool is formed and that protein is synthesized. It was also found that washing the proto-

plasts on membrane filters with isotonic media removed the labeled amino acid pool without causing lysis. Thus the amino acid pool is held so loosely that even mechanical mishandling of the protoplasts causes its loss.

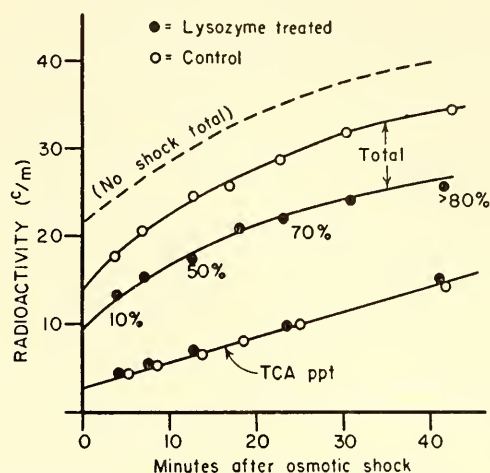


Fig. 32.  $C^{14}$ -proline utilization by *E. coli* after osmotic shock in the presence of lysozyme (solid circles). Percentages indicate the proportion of cells that were spherical as determined by direct observation in the phase-contrast microscope. Lysozyme was omitted in the control culture (open circles). The larger pool size for cells that received no shock or lysozyme treatment is shown by the dotted line.

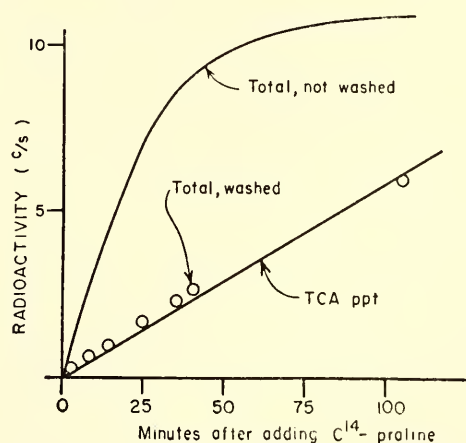


Fig. 33.  $C^{14}$ -proline utilization by a suspension of cells containing 96 per cent spherical forms (solid lines). Washing with isotonic medium removes the pool but does not lyse the cells (open circles).

#### FRACTIONATION OF CELL JUICES ON ION-EXCHANGE COLUMNS

Proteins and nucleic acids of *E. coli* have been separated and characterized by means of cellulose ion exchangers.<sup>1</sup> Small amounts

<sup>1</sup> For preparation of the exchangers see E. A. Peterson and H. A. Sober, *J. Am. Chem. Soc.*,



of TCA-soluble bacterial amino acids, which occur in apparent association with high-molecular-weight components of cell juice, have also been isolated by means of the exchanger. Various proteins having different enzyme activities have been separated.  $\beta$ -Galactosidase activity has been found distributed among different proteins separated by the exchanger. Bacterial nucleic acid has been separated from bacterial protein, and the nucleic acid has been further fractionated.

*Association of bacterial amino acids with high-molecular-weight components of E. coli.* It has become evident from studies on protoplasts that the pool holding capacity of the *E. coli* cell lies beneath the cell wall. Normal pools are still formed when the cell wall has been fundamentally altered by lysozyme and osmotic shock. Unfortunately, the inner architecture of the cell is only crudely known. There is not yet completely satisfying evidence of the physical reality of a plasma membrane or of a nucleus. On the other hand, it is clear that much of the substance of the cell is organized into macromolecules or complexes of macromolecules such as the 20 S and 40 S ribonucleoprotein particles; see figure 30. An attempt was made, therefore, to learn whether pool amino acids could be found in association with macromolecular components.

For this purpose the experimental procedure was as follows. Exponentially growing *E. coli* were suspended in 0.5 M sucrose dissolved in Tris medium, and were disintegrated with a modified French pressure cell. The resulting suspension was centrifuged, the pellet was discarded, and the supernatant fluid was filtered through collodion membranes to yield a clear amber cell juice. This juice contains all the material of the original suspension except the cell walls and a small amount of unbroken cells. To the juice was added a solution of carrier-free  $C^{14}$ -labeled alanine,

glutamic acid, leucine, and isoleucine for the purpose of (a) tracing the behavior of any free amino acid of bacterial origin, and (b) distinguishing bacterial amino acids that were not free to equilibrate with the  $C^{14}$ -labeled substances under these conditions. This radioactive mixture was immediately passed into a column of the anion exchanger DEAE cellulose (diethylaminoethyl cellulose).

Washing the column with several column volumes of 0.25 M sucrose in Tris medium completely removes the  $C^{14}$ -labeled amino acids, the bulk of the bacterial amino acid, and a small fraction of the protein and nucleic acid. Elution with 1 M NaCl dissolved in the sucrose-Tris medium releases nearly all the remaining protein and nucleic acid from the column. When the latter substances and salts are removed from the eluate by TCA treatment and ion exchange, amino acids can be recovered and identified by paper chromatography (fig. 34). These amino acids are found to be nonradioactive. Thus, they are clearly of bacterial origin but differ in their behavior on the ion exchanger from the bulk of the TCA-soluble bacterial amino acids and also from the admixed  $C^{14}$ -tracer amino acids. In quantity they correspond to a few per cent of the pool amino acids originally present in the cells. A definite tie or association between these amino acids and macromolecules seems the most probable explanation for this behavior.

Depending on the criterion employed, these associated amino acids resemble or differ from the metabolic pool amino acids of *E. coli*. For example, like the metabolic pool amino acids, they cannot be recovered from cells that have been osmotically shocked by washing with distilled water. Alternatively, when cell juice is prepared in 0.5 M sucrose solution lacking salts, and then passed over the ion exchanger, the free neutral amino acids pass through the column. The anionic amino acids, glutamic and aspartic acids, and also a small amount of neutral amino acids of bacterial origin, however, remain adsorbed. These

78, 751 (1956); for application to plasma protein separations, see H. A. Sober et al., *J. Am. Chem. Soc.*, 78, 756 (1956).

neutral amino acids can be released by washing the column with distilled water and be identified chromatographically (fig. 35). Proteins and nucleic acids are not released by this treatment. Thus, the ion-exchanger-macromolecule complex holds the associated amino acids in a labile fashion reminiscent of the way in which the metabolic pool amino acids are held in the cell. On the other hand, the macromolecule-associated amino acids differed in that they could not be labeled by exchange with

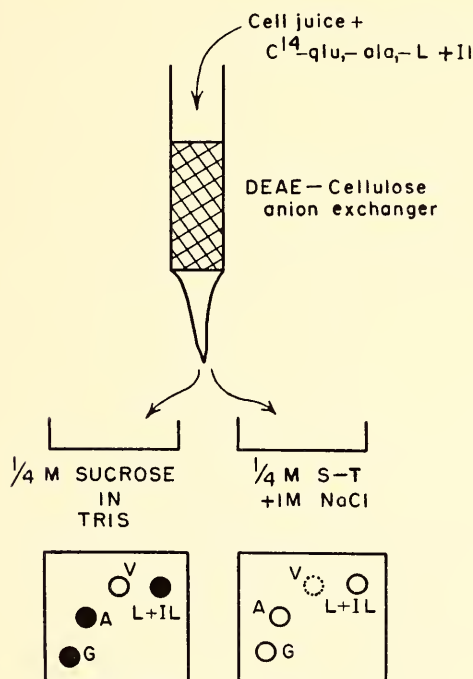


Fig. 34. Ion-exchange separation of bacterial amino acids from admixed  $C^{14}$  amino acids. Solid circles indicate the location of labeled amino acids on two-dimensional paper chromatograms; open circles indicate unlabeled amino acids. A=alanine, G=glutamic acid, V=valine; L+IL=leucine, isoleucine.

$C^{14}$  amino acids, by using either cell juices, as described above, or undamaged cells at  $0^\circ C$ .

It is not yet clear what role the macromolecule-associated amino acids play in the metabolism of the cell. Although they constitute only a few per cent of all the TCA-soluble amino acids in the cell, they may represent a transitional phase lying between the metabolic pool amino acids and the protein end product.

*Protein and nucleic acid fractionation on*

*cellulose ion-exchange columns.* Several examples of the separation of *E. coli* proteins and nucleic acids are shown below. Figure 36 illustrates the separation of  $S^{35}$ -labeled components of pressure cell juice prepared from cells grown to a high density from a small inoculum in the presence of  $S^{35}O_4^{2-}$ . The effluent volume is given on the abscissa, and the amount of radioactivity (upper line) or amount of protein (lower line, chemically determined with the Folin phenol reagent), on the ordinate. It is evident that the radioactivity and the

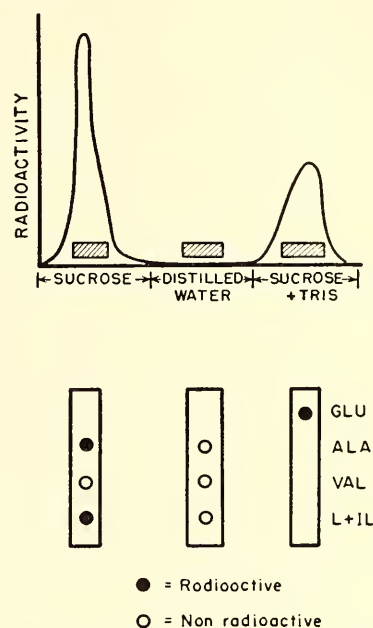


Fig. 35. Separation on DEAE cellulose of bacterial amino acids from  $C^{14}$ -labeled admixed amino acids by means of distilled-water washing. The amount of  $C^{14}$  eluted is plotted against the volume of eluent. Fractions shown as shaded areas at the centers of each region were chromatographed on paper in one dimension. The result is depicted immediately below the corresponding shaded area.

protein of the cell can be resolved into several components. The largest  $S^{35}$  peak is most probably due to the peptide glutathione; the remaining peaks are due to proteins. Comparison of the specific radioactivities for several of the components of the diagram demonstrates that the components differ in the amount of sulfur that they contain relative to each chemical unit (principally the amino acid tyrosine) which reacts with the phenol reagent.



Thus, different proteins are separated. Ion-exchange separations of the proteins of cells exposed to  $S^{35}O_4^{=}$  for only a few minutes reveal several regions of even higher and several regions of lower specific radioactivity. Such a finding bears the implication that the sulfur of some kinds of proteins passes into other proteins. The ion exchanger, thus, provides a tool for the study of possible generic relationships among the bacterial proteins.

a separation is shown in figure 39. There is little correlation between the nucleic acid (ultraviolet absorption at 260 m $\mu$ ) distribution and the protein distribution ( $S^{35}$  content). Since ultracentrifuge and electron-microscope studies have shown that much of the ribonucleic acid is found in spherical particles of about 1 million molecular weight it appears that these cellulose ion exchangers, as we have employed them, destroy the original nucleo-

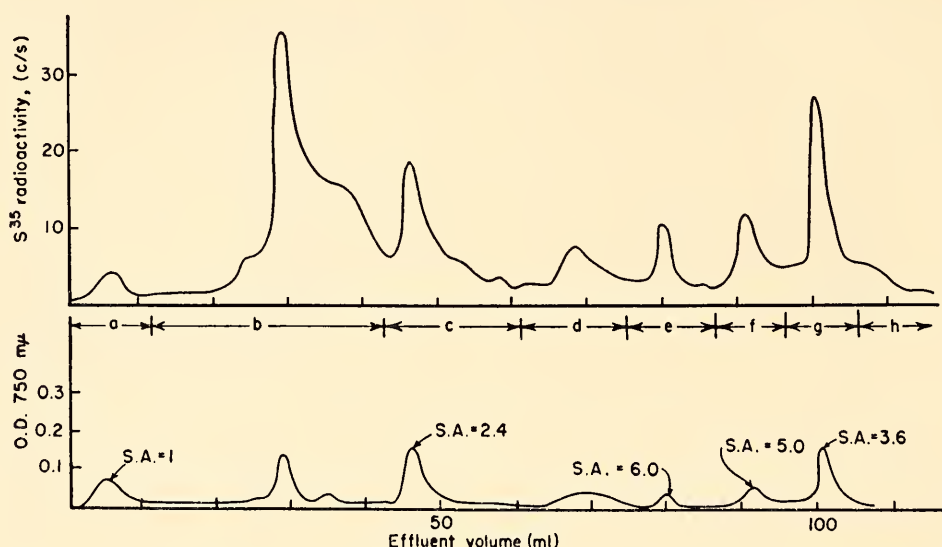


Fig. 36. Elution diagram of  $S^{35}$ -labeled components and proteins of *E. coli*. One-milliliter effluent samples were analyzed for each region. The elution schedule was as follows:

a=	0.5 M	sucrose					
b=	"	"	+0.05 M	Tris-succinate	pH 7.7		
c=	"	"	+0.1 M	"	"	"	
d=	"	"	+	"	"	pH 7.0	
e=	"	"	+	"	"	"	+0.05 M NaCl
f=	"	"	+	"	"	"	+0.1 M "
g=	"	"	+	"	"	"	+0.2 M "
h=	"	"	+	"	"	"	+0.4 M "

Figure 37 illustrates the separation of two enzyme activities, acetokinase and  $\beta$ -galactosidase.

Higher resolution among the protein components can be achieved by eluting the column with a smoothly increasing concentration of salt as shown in figure 38. In this run, activity corresponding to the adaptive enzyme,  $\beta$ -galactosidase, was found in a well defined region of 120 to 150 ml effluent volume.

Nucleic acids may also be separated from one another and from protein by means of cellulose ion exchangers. Such

protein associations. It should be possible, therefore, to use the ion exchangers to determine the complexity of subcellular particulates such as the microsomal particles.

#### INCORPORATION OF AMINO ACID ANALOGUES INTO PROTEINS

Through collaboration with Dr. Georges N. Cohen, of the Institut Pasteur, it has been shown that selenomethionine can completely replace methionine for exponential growth of a methionine-requiring mutant of *E. coli*. Active  $\beta$ -galactosidase is found under this condition. Experiments

are in progress to find whether this  $\beta$ -galactosidase has the same specific molecular activity as the normal enzyme.

The demonstration that exponential growth occurs when selenomethionine replaces protein methionine reflects the synthesis of all the essential enzymes. Workers at the Institut Pasteur have found that,

with other structural analogues of amino acids (*p*-fluorophenylalanine,  $\beta$ -2-thienylalanine, norleucine), linear growth, instead of exponential growth, always occurred, accompanied by the suppression of the synthesis of one or more active essential enzymes. In each case the analogues were incorporated into the proteins, replacing structurally related natural amino acids. These results and the selenomethionine data demonstrate that the amino acid composition of protein can be altered by the presence of amino acid analogues in the medium. Since the incorporation of amino acid analogues into protein results either in the synthesis of fully active enzymes or in the suppression of synthesis of some enzymes, the analogues become powerful tools in the study of the protein-forming mechanisms.

*Incorporation of selenium into proteins.* While selenomethionine can completely replace methionine, selenium cannot entirely replace sulfur for growth of *E. coli*; but, when the cell contains glutathione, which can serve as an internal sulfur reservoir, or when traces of exogenous sulfate are added, selenium partially replaces sulfur. Hydrolysates of proteins obtained from cells grown in media containing radioactive selenite and trace quantities of sulfate are found to contain a radioactive material with chromatographic properties similar to those of cysteine. The incor-

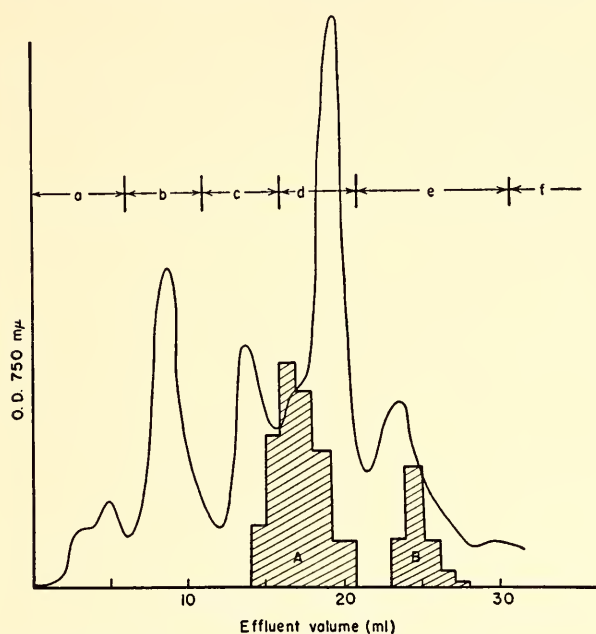


Fig. 37. Ion-exchange separation of acetokinase (A) and  $\beta$ -galactosidase (B). The total protein eluted is shown as a continuous line. The elution schedule followed was:

a=	0.02 M	$\text{PO}_4^{3-}$	+ 0.005 M	cysteine	+ 0.0 M	NaCl
b=	"	"	"	"	0.1 M	"
c=	"	"	"	"	0.2 M	"
d=	"	"	"	"	0.3 M	"
e=	"	"	"	"	0.4 M	"
f=	"	"	"	"	0.6 M	"

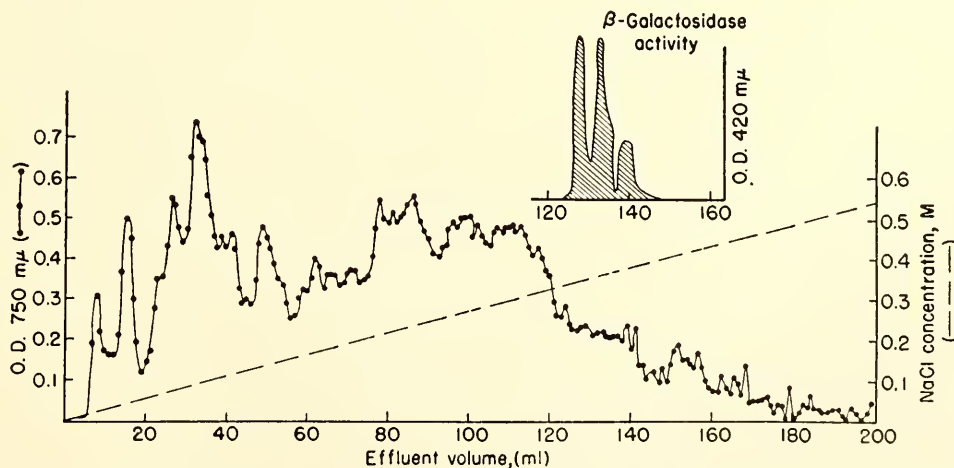


Fig. 38. Elution diagram of *E. coli* protein on DEAE-cellulose column. A linear salt gradient was used as shown on the broken line. The protein pattern (continuous line) is very complex, and  $\beta$ -galactosidase (insert) has been resolved into three apparent components.



poration of selenium is proportional to the increase in bacterial mass. Selenium is also incorporated into the alcohol-soluble proteins, but, unlike the sulfur of this protein, cannot be transferred from it to the residual proteins during sulfur starvation.

A possible reason for the inability of selenium to totally replace sulfur for the synthesis of proteins might be the inability of selenocysteine to form Se-S or Se-Se bridges, or the incompatibility between proteins containing such linkages and a

diameter) is the most prominent. A second group, which is sometimes resolved into two groups, has roughly one-half this value.

As it appeared possible that one group of particles might be the product of the other, an attempt was made to observe kinetic relationships among the groups. Cells were labeled with  $S^{35}$  and  $P^{32}$  during a long exposure to the tracer to give a steady-state condition, or during a short exposure to place the tracer materials in

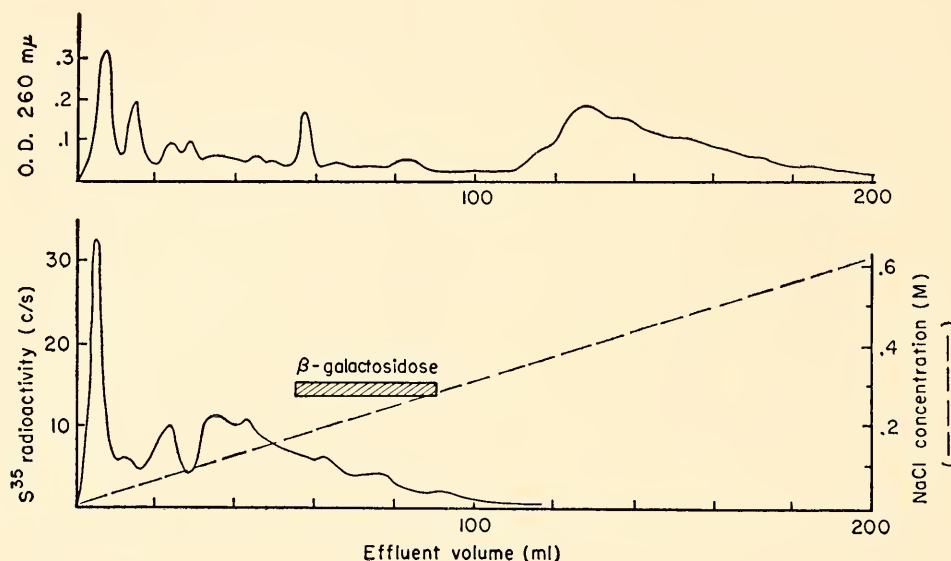


Fig. 39. Elution diagram of nucleic acid (O.D. at 260  $m\mu$ ) and protein ( $S^{35}$  radioactivity) on an Ecteola-cellulose column. A linear salt concentration gradient was used for elution. The occurrence of  $\beta$ -galactosidase is shown by the shaded area.

normal catalytic activity. It is also possible that the responsible enzymes of *E. coli* are incapable of transforming selenocysteine to selenomethionine. This possibility is strengthened by the finding that seleno-glutathione is not formed from selenite. Perhaps some of the biosynthetic enzymes might thus show a more strict specificity than the protein-synthesizing mechanism.

#### PARTICLES

Various lines of evidence point to the importance of small particles in the synthetic activities of the cell. Sedimentation analysis shows several groups of particles in the juice of *E. coli*. In most of the published work a group having a sedimentation constant of 40 S (corresponding to a molecular weight of about 1 million, 200-A

the early product of synthesis. The cells were then broken, and the unbroken cells and cell walls were centrifuged out. The supernatant fluid containing the small particles was then centrifuged at 105,000g for various periods of time. The cellular material was separated into cell walls, particles, and nonsedimentable material, but no separation of the groups of particles was achieved. Different runs gave quite different rates of sedimentation; the particles seem very sensitive to the composition of the suspending fluid.

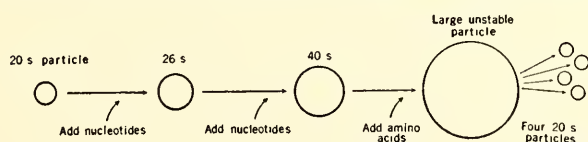
It did appear that, when the tracer was supplied for a short time only, the specific radioactivity of the fraction containing the particles was higher than that of the other fractions. Also, chromatography of the particle fraction showed that a small but

definite portion of the amino acid pool is carried down with the particles. Further work is planned for next year to establish the kinetic relations between the particles and the other parts of the cell.

### Model

During the year we have attempted to arrange the information about the activities of the cell in terms of definite models. Even if they are completely wrong, models are of some value, as they suggest numerous experiments. The models attempt to interpret two known features of the cell's operation: the role of particles in synthesis, and the role of organization in the cell.

A large fraction of the cell mass is found in the form of ribonucleoprotein particles. Most of the RNA of the cell and a considerable fraction of the protein can be centrifuged down; indeed, it seems quite possible that most of the protein and RNA of the cell is associated with particles, some of the protein being tightly bound and some being released during the process of disrupting the cell. Accordingly, the synthesis of a new cell involves as a major activity the synthesis of new particles. In one model we have interpreted the different classes of particles observed in the ultracentrifuge (20, 26, and 40 S) as stages in a process wherein small particles grow to large particles which then disintegrate into several small ones. Such a model emphasizes the necessity for experiments to determine the composition and kinetic relations of the different classes of particles.



According to this model, each particle would synthesize and contain only one type of protein. Therefore, the organization of enzymes must result from a spatial organization of the particle with respect to other particles. The cell wall does not provide enough area to hold the particles. Consequently, it seems reasonable to as-

sume that they might be held in a definite framework by the deoxyribose nucleic acid. Calculation shows that there is sufficient DNA to provide a framework with the DNA arranged in coils (300-A diameter, 200-A pitch) with their axes parallel. Such a structure could account for the relationship between the order of genes and the order of biochemical events which was reported last year by the Department of Genetics of the Institution. It also appears possible for such a structure to duplicate all its individual components and then to divide by extrusion into two identical structures.

This model summarizes our present knowledge of the cell. It shows that we are beginning to relate structure and function, but it also emphasizes how little is known of either.

### Hoagland Compound

During the past year a compound has been reported by Dr. Hoagland in which amino acids are linked to nucleic acid. It is reasonably stable in acid but unstable in basic solution. Such a compound would appear in the "TCA-precipitable" fraction and might be mistaken for protein. Tests were therefore made to determine how much of this compound is present in growing *E. coli*. A radioactive amino acid was added to a growing culture of cells, and the usual samples were taken to measure the total and TCA-precipitable fractions. In addition, samples were injected into NaOH to release any amino acids bound to nucleic acid. The samples were then neutralized and mixed with TCA to precipitate protein. In *E. coli* this procedure showed that only a minute fraction of the amino acids could be bound in alkali labile compounds. With carrier-free amino acids and washed cells from which all pre-existing pools were removed there was an indication that such compounds were present, but only in quantities of about 1  $\mu\text{M/g}$  cells. The usual pool would be about 100  $\mu\text{M/g}$ , and the amino acids in the proteins, of course, would amount to several thou-



sand micromoles per gram of cells. In yeast (*C. utilis*) a considerably greater fraction showed the characteristics expected of amino acids bound to nucleic acid. The quantity in this case was large enough (15  $\mu$ M/g dry cells) to offer an opportunity for its isolation and for further study of its kinetic characteristics.

### *Protomorphs*

For some time it has been apparent that organization is important in the bacterial cell. The cell is by no means a "bag of enzymes"; disruption of the cell may have little effect on individual enzymic activities, or even may increase them, but many of the more elaborate functions that require the co-operative action of several enzymes cease. In particular, crushing cells greatly reduces their ability to incorporate amino acids into protein.

It is also highly probable that the organization of the cell stems from a spatial arrangement of the constituent molecules. The localization of many of the Krebs cycle enzymes into the mitochondria of mammalian cells has been observed. A large part of the bacterial protein and ribonucleic acid is organized into microsomal particles, and similar particles have been found to play a major role in protein synthesis in mammalian cells. In mammalian cells the microsomal particles are arranged on the surfaces of membranes; in the bacterial cell it is likely that they are also held in a definite spatial arrangement, perhaps in a framework of deoxyribose nucleic acid.

Since, presumably, the forces responsible for these organized systems are properties of the macromolecular constituents of the cells, it seems possible that thoroughly disorganized cellular material might, under the proper conditions, reconstitute some of the spatial arrangements of the cell analogously to the reconstitution of tobacco mosaic virus from its protein and RNA components.

Having these thoughts in mind, we were very much interested to observe the de-

layed appearance of particles in a clear preparation of cellular juices. These particles have several properties that distinguish them from coacervates formed from the gelatin and nucleic acid; it even seems possible that they offer a new form for study which is intermediate between the highly organized cell and disorganized cell juice. Because they are formed from protoplasm and have a distinctive shape, it seems suitable to refer to them as "protomorphs," to avoid confusion with the many other particles of cellular origin.

*Preparation.* These particles develop in a preparation made as follows: Wash 10 g of *E. coli* growing in a glucose mineral salts medium (C) with a solution (TSS) 0.25 *M* in sucrose buffered with trishydroxymethylaminomethane (Tris) 0.01 *M* and brought to pH 8 with succinic acid. Centrifuge out the cells, and resuspend them with 10 ml TSS. Break the cells by forcing the suspension through a small hole at 10,000 psi. Bring the volume up to 50 ml with TSS, and centrifuge 10 minutes at 40,000*g*. This treatment removes any unbroken cells and the larger fragments of cell walls. Decant the supernatant fluid, and centrifuge at 105,000*g* for 15 minutes to remove the smaller fragments of cell walls. Again decant the supernatant fluid, which should be quite clear, and dilute to 100 ml with TSS. Add  $\text{MgCl}_2$  and  $\text{MnCl}_2$  to make the solution 0.005 *M* in each. Leave overnight, and the originally clear solution becomes quite cloudy owing to the formation of protomorphs. The yield is variable but may be as high as 0.5 g. The pH of the solution must be 7 to 8; below pH 7 the protomorphs do not form, and below pH 6 a precipitate develops when the manganese is added. All operations following the breaking of the cells are performed at 0° to 5° C.

In this procedure some variation is probably permissible; only a few of the variables have been studied. The addition of manganese is essential; magnesium is not essential but improves the yield. The purpose of the low temperature during the

formation period is to reduce bacterial contamination and denaturation of protein. When the cell juice is centrifuged for  $2\frac{1}{2}$  hours at  $105,000g$  to remove the microsomes (absorption at  $260\text{ m}\mu$  reduced by 67 per cent), it gives a much lower yield of protomorphs. The microsome pellet when resuspended in TSS, however, gives a precipitate but no protomorphs on addition of manganese. Other variables, such as dependence on the presence of Tris, succinate, or sucrose, have not been investigated.

*Appearance, composition, stability.* The protomorphs formed by this procedure appear as shown in figure 40, plate 5. (Our thanks go to Dr. Wm. Duryee for making this photomicrograph.) They are very nearly spherical, and range in diameter from about 1 to 5 microns. Estimated by the absorption at  $260\text{ m}\mu$  (1 mg nucleic acid/ml gives optical density=25), the nucleic acid content is roughly 20 mg/g wet weight. The Folin reaction indicates that there is approximately 50 per cent more protein per unit of  $260\text{-m}\mu$  absorption than in the cell juice from which the protomorphs formed; thus, the protein content is roughly 120 mg/g wet weight. Paper chromatograms run in butanol/formic acid/water show the presence of lipid material in about the same proportion as appears in preparations of microsomes from *E. coli*. Thus, the protomorphs contain several of the constituents of bacterial protoplasm.

In contrast to simple coacervate particles, which can exist only in a narrow range of  $pH$ , the protomorphs are quite stable. They can be centrifuged down and resuspended without disintegrating; they maintain their form in  $H_2O$ , 1  $M$   $NH_4OH$ , or 5 per cent trichloroacetic acid (TCA), or after shaking with ether. Sodium phosphate buffer (0.07  $M$ ,  $pH$  7) dissolves out much of the nucleic acid of the protomorphs, leaving a less optically dense residue; casein hydrolysate (5 per cent) has much the same effect. Ethylenediaminetetraacetic acid (EDT) (0.01  $M$ ) dis-

solves the protomorphs, leaving a clear solution.

*Functions of the protomorphs.* Suspensions of the protomorphs in TSS supplemented with  $5 \times 10^{-3} M$  Mg were incubated with  $P^{32}O_4$  ( $4 \times 10^{-5} M$ ) at  $22^\circ C$ . Samples were taken periodically and filtered. Other samples were mixed with equal volumes of 10 per cent TCA and subsequently filtered. The concentration of  $P^{32}O_4$  in the protomorphs increased steadily with time; all this phosphate was extracted with TCA, however, and there was no sign of incorporation of  $P^{32}$  into nucleic acid.

Similar experiments were carried out using a mixture of  $C^{14}$ -labeled amino acids from hydrolyzed *Chlorella* protein. A steady increase in the  $C^{14}$  content of the protomorphs was observed. In this case, however, the major portion was not extracted with TCA.

As this incorporation rate is very small compared with that of bacteria, it is necessary to show that bacterial contamination could not cause the incorporation. Plate counts and microscopic examination of the culture indicated that the bacteria present could at most account for only 1 per cent of the observed incorporation.

Chemical tests were made to determine whether the radioactivity of the TCA-insoluble material was bound in protein. The radioactivity was precipitable with TCA after the insoluble material was dissolved in 1  $N$  NaOH. On paper chromatograms the radioactivity had the same movement as protein; after hydrolysis it was found with the free amino acids.

Clearly, from this preliminary report, much work remains to be done to establish the nature of these protomorphs. Both the yield and the synthetic ability are highly variable from one experiment to another. Not only do the conditions of formation require further exploration, but also the functional properties must be investigated in more detail. It is not certain whether the incorporation of amino acids represents net synthesis or some form of exchange.



The protomorphs may have a functional performance inherently different from the disorganized cell juices from which they are formed, or they may merely represent the separation of functional material from some inhibitory substance in that medium. Studies are in progress that should clarify these points.

#### PROTEIN SYNTHESIS IN MOUSE TISSUES

During the year our collaboration with Drs. L. B. Flexner and J. B. Flexner, of the University of Pennsylvania, has continued. Observations on amino acid and protein synthesis in the liver and cerebral cortex of the mouse have been extended to include adult as well as newborn animals. Kinetic data obtained from 4 or 5 litter-mates sacrificed at various times up to 1 or 2 hours after injection of glucose or amino acids labeled with  $C^{14}$  showed the concentrations of labeled glucose in blood and tissues and the concentration of labeled amino acids in blood, tissue pool, and tissue protein.

One of the unanticipated and striking findings is the high rate of utilization of carbon of glucose for synthesis of nonessential amino acids in the cerebral cortex. A complete, quantitative analysis of the contribution made by all sources to any one of these nonessential amino acids is lacking. The importance of glucose, however, is made evident by the finding that it supplies ten times more carbon to glutamic acid and glutamine of cortex than does blood glutamic acid. This is true for both the newborn animal and the adult. In contrast, the quantities of glutamic acid derived from glucose by the liver of newborn and adult is about the same as that derived from blood glutamic acid. In both tissues, the rate of incorporation of glucose carbon into carbon of amino acids is far higher in the adult than in the newborn, and correspondingly the rate of degradation of the amino acids is higher in the adult than in the newborn.

At present, a major concern of this work is to gain a measure of the relative rates

of protein synthesis and degradation at different stages of growth. The nerve cells of the cerebral cortex of the newborn mouse must synthesize protein to provide both for the increase in cell size and for the increase in number of nerve processes that accompanies development. The nerve cells of the adult animal, however, have reached their final size; they do not divide; and consequently they must synthesize protein only to compensate for that lost by degradation. Much the same basic difference exists between the liver of the newborn and that of the adult, although in this organ the situation is complicated by the demands made upon it for the synthesis of plasma proteins of the blood.

The experimental observations made so far permit a first step in analyzing the effect of growth and degree of maturation on rate of protein synthesis. Calculations have been made of the rates at which individual amino acid pools furnish their amino acids for protein synthesis. There is a surprisingly small difference between the newborn and adult animal. In the cerebral cortex, the pools of the newborn are drawn upon at only twice the rate of the adult. In the liver even less difference between the two age groups is found. Further analysis of these findings in terms of protein synthesis depends upon determination of the concentrations of individual amino acids in their respective pools.

#### VISITORS

During the year we benefited by a number of visitors who worked with us in the laboratory for short periods of time. They included Dr. Julius Marmur, Harvard University; Mr. Charles Stroebel, University of Minnesota; Dr. J. R. Vallentyne, Geophysical Laboratory; Mr. Ellis Kempner, Yale University; Mr. S. K. Roberts, Princeton University. Co-operative work was carried out with Dr. S. Bernhart, Naval Medical Research Institute; Dr. Louis Flexner, University of Pennsylvania; and Dr. Bill H. Hoyer and Dr. Edgar Ribi, of Rocky Mountain Laboratory, U. S. Public

Health Service. Dr. F. T. McClure, Applied Physics Laboratory, Dr. G. Cohen, Pasteur Institute, and Dr. John Leahy, who were here as part of the Fellowship

Program, contributed greatly to the biophysics section. We also wish to thank several members of the National Institutes of Health for the use of their equipment.

## OPERATIONS AND STAFF

### *CO-OPERATIVE WORK OF THE DEPARTMENT*

The Institution's policy of co-operation has been continued with institutions in this country and abroad, including the Applied Physics Laboratory, Associated Universities, Inc., Bernard Price Institute of Geophysical Research (Africa), Brookhaven National Laboratory, Chalmers University of Technology (Sweden), U. S. Coast and Geodetic Survey, Department of Defense, Geological Survey of Canada, U. S. Geological Survey, Geophysical Institute of Huancayo (Peru), Johns Hopkins University, Lamont Geological Observatory, Mount Wilson and Palomar Observatories, National Bureau of Standards, National Institutes of Health, National Research Council, National Science Foundation, Ontario Department of Mines (Canada), Pasteur Institute (France), Rocky Mountain Laboratory of U. S. Public Health Service, Universities of Missouri, Pennsylvania, and Virginia, University of Melbourne (Australia), and Yale University. We continued our collaboration with the American Geophysical Union, the International Union of Geodesy and Geophysics, and the International Scientific Radio Union. Foreign and domestic visitors have used our facilities, some on fellowships of the Institution from England, France, and various parts of the United States.

Research work concerned with mineral ages using isotope measurements was continued with the Geophysical Laboratory.

We have had continued assistance in our cosmic-ray investigations from the observatories at Cheltenham, Maryland (to September 30, 1956); Christchurch, New Zealand; Climax, Colorado; Fredericksburg, Virginia (from October 1, 1956); God-

havn, Greenland; Huancayo, Peru; and Mexico, D. F.

Contracts with the government (no overhead charges) have been continued for the investigation of the earth's crust and of cosmic rays. Licenses are in force with the Atomic Energy Commission for the procurement of isotopes for nuclear studies, measurement of mineral ages, and biophysical investigations.

Three grants from the National Science Foundation for the activities of the NSF Advisory Panel on Radio Astronomy are being administered, without charge.

The National Science Foundation has also provided funds for the partial cost of projects, to be carried out in South America as our participation in the International Geophysical Year, concerned with seismic crustal investigations and with the height in the ionosphere of equatorial electrojets.

We have supplied ionium collectors to the National Meteorological Service of Argentina and to the Instituto Geofísico de Universidade do Porto of Portugal.

Several staff members have continued their service on panels of the U. S. National Committee of the International Geophysical Year, and one is a member of the Executive Committee of that Committee; until December 31, 1956, one member continued full time with the National Academy of Sciences on studies concerning a world data center in connection with the International Geophysical Year. One member continues as Chairman of the Advisory Panel on Radio Astronomy of the National Science Foundation. Two members are on the Committee on Growth of the National Research Council, and one is on the Council of the Biophysical Society and continues to serve on the Advisory Committee



to the Federal Civil Defense Administration. One member continues as Chairman of the U. S. A. National Committee, International Scientific Radio Union. Another staff member (resigned December 31, 1956) continued his full-time research work for the government.

One staff member visited many institutions abroad on a world trip sponsored by the Carnegie Corporation of New York. Another participated in an electrojet survey in South America as a part of one of the Department's projects for the International Geophysical Year. Another member spent several months at the Pasteur Institute in Paris. One staff member is abroad on a Guggenheim Memorial Fellowship.

Department staff members attended the Twentieth International Geological Conference in Mexico City, the symposium on Polar Atmosphere at Oslo, Norway, the International Conference on Rock Magnetism in London, the Geophysics Colloquium at Cambridge, England, and the symposium on Electromagnetic Phenomena in Cosmical Physics at Stockholm, Sweden.

#### ADMINISTRATION AND OPERATION

The Department continues to publish the *Journal of Geophysical Research*, partly subsidized by the Institution.

We have continued to rent part of one farm for the activities of the radio astronomy group.

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*Machinist:* F. J. Caherty.

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Fig. 1. A portion of the 330-mc helix array used for locating sources of radio radiation on the solar disk. The two helical receiving elements are mounted on a common ground screen and are connected to an open-wire transmission line. The present array contains 30 units like the one shown above and occupies a line 2000 feet long at the River Road field station.



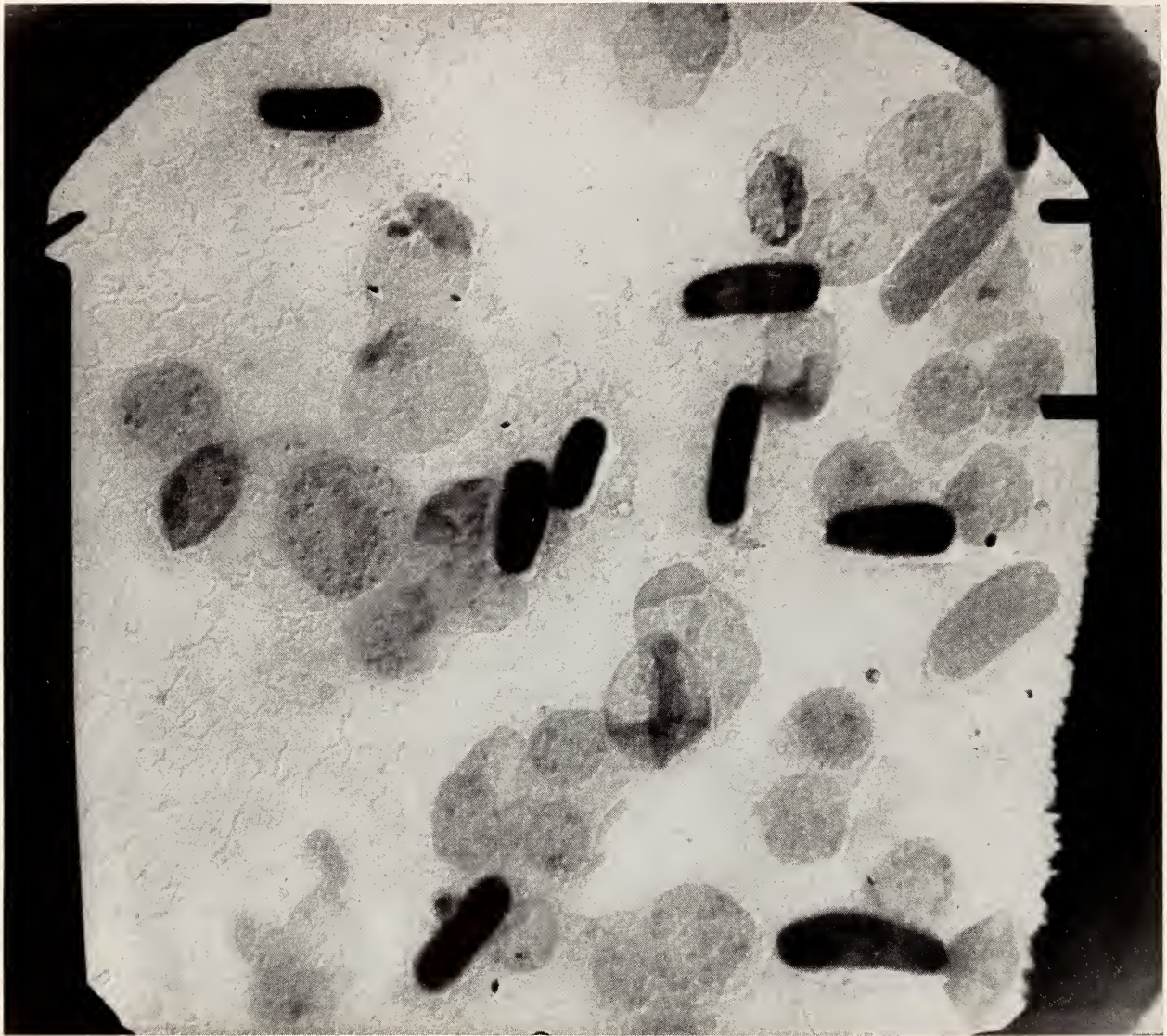


Fig. 29. Electron micrograph of cell walls from *E. coli* treated with lysozyme and osmotically shocked. The walls appear as flattened disks which show a high incidence of double structure as though the original condition were of the bag-within-a-bag type. The dark rods are unbroken bacteria about 1.5 microns long.

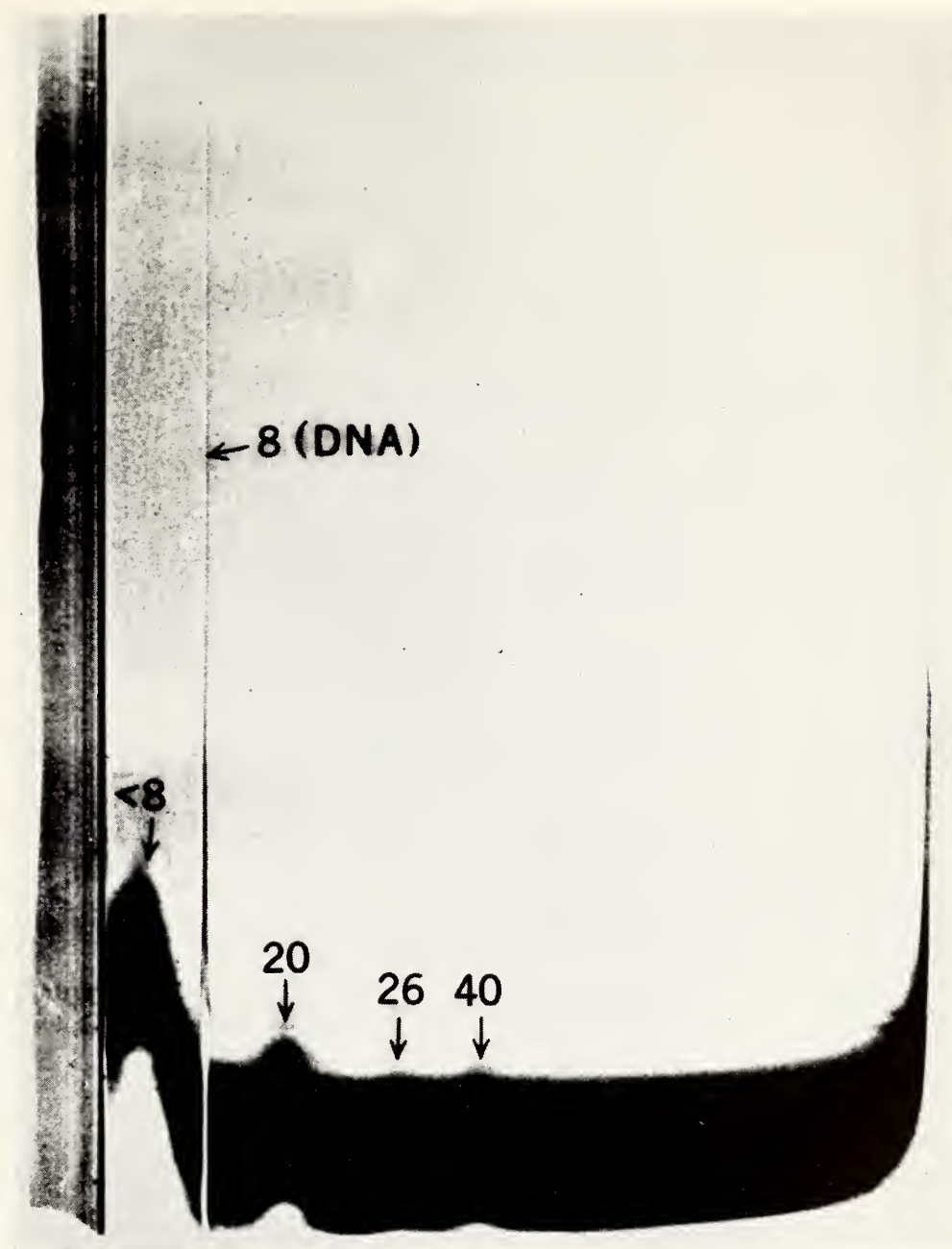


Fig. 30. Sedimentation diagram of *E. coli* extract prepared by lysozyme treatment and osmotic shock. The picture was taken about 30 minutes after the rotor reached a speed of 59,000 rpm. The most obvious components are the constituents labeled 40 S, 26 S, 20 S, and 8 S. The sharp spike is due to deoxynucleic acid. Such a simple pattern contrasts sharply with the exceedingly complex diagrams of the protein composition of *E. coli* as revealed by ion exchange (cf. fig. 36).



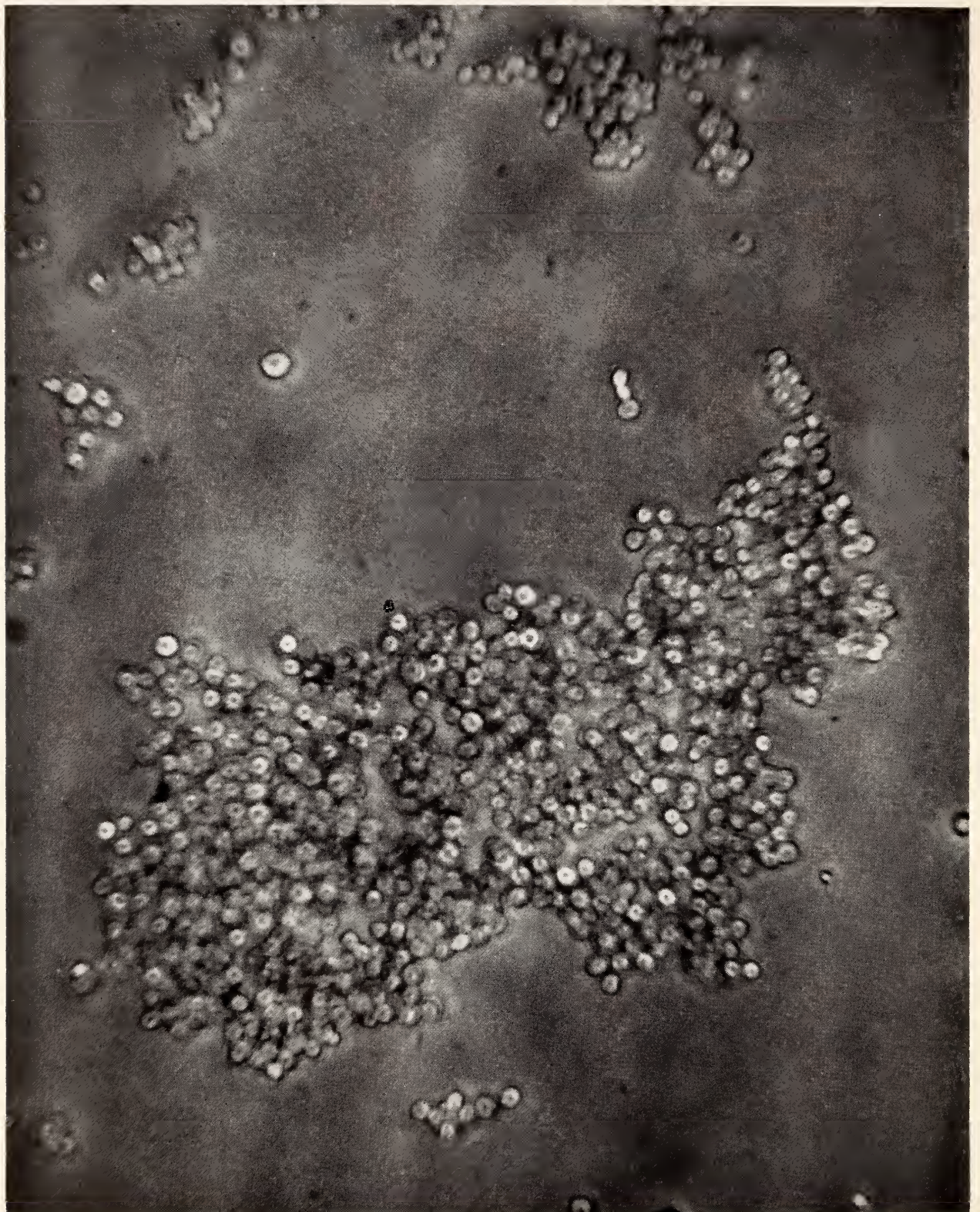


Fig. 40. Photomicrograph of aggregated protomorphs (magnification,  $\times 330$ ).



# G E O P H Y S I C A L   L A B O R A T O R Y

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*Washington, District of Columbia*

PHILIP H. ABELSON, *Director*



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## INTRODUCTION

The Geophysical Laboratory continues as one of the active centers of research in earth science. Through application of the tools and viewpoints of physical science we are achieving new understanding of the nature and history of the processes by which the earth evolved to its present state.

The principal lines of activity at the Laboratory are studies of phase-equilibrium relations of the major mineral groups, research on ore minerals, radioactive age measurements, paleobiochemistry, chemical reactions at extremely high pressures, crystallography, and investigations of order-disorder phenomena.

Phase-equilibrium relations among the major mineral groups are providing a whole series of geological "thermometers" which may be applied to igneous and metamorphic rocks, furnishing tools for acquiring a great deal of information about the conditions under which such processes as mountain building occurred. Similar types of thermometers are being developed for ore minerals. The ultimate accumulation of a number of these will permit cross checks and reliable determinations of the temperatures present during ore formation, which, in turn, will lead to better understanding of the processes involved and inevitably to knowledge having economic importance.

The co-operative radioactive dating program continues to open new vistas. Reliable dates may now be assigned to events occurring in any age throughout the earth's

history. Studies in paleobiochemistry potentially may lead to new and detailed understanding of the origins and development of life as well as to a better grasp of such problems as the origins of petroleum. When equipment currently under development is operable, this Laboratory will be able to participate very actively in advancing one of man's great frontiers—high-pressure studies. It is abundantly clear that a new type of chemistry awaits discovery and exploitation.

Crystallography is becoming one of the most vital fields in science today. By means of neutron diffraction, nuclear and paramagnetic resonance, and X-ray diffraction, the structural chemist is learning how the precise determination of molecular arrangement leads to real understanding of chemical reactivity. The nature of the chemical bond as manifested in covalent, metallic, ionic, or intermediate types is closely related to structure.

During the past year Chayes has achieved what may be a breakthrough in understanding various types of order-disorder in crystals. Using an optical analogue he has produced diffraction patterns similar in type to those obtained from X-ray studies of crystals. It is too early to evaluate the practical consequences, but fundamental knowledge in such an area as the physics of the solid state will certainly lead to useful applications. Detailed description of Chayes' studies and of other work of the Laboratory follows.

## ORDER AND DISORDER

*F. Chayes*

### EVIDENCE FOR ORDER AND DISORDER IN MINERALS

Striking differences in physical properties are often noted in metals or metallic compounds, depending on whether annealing has taken place above or below some critical temperature. X-ray diffraction patterns may also differ markedly with an-

nealing temperatures, and it was largely the interpretation of these latter differences that led to the crystallographic superlattice and the closely related notion of long-range ordering. The crystallographer's superlattice in turn led directly to the "interpenetrating-lattice" models of ordering used in statistical thermodynamics, in which long-



range ordering is usually treated as an end product of short-range ordering.

Recent progress in experimental petrology has focused attention on solid-state transitions, and in the course of the last decade mineralogists and petrologists have gradually adopted much of the viewpoint and vocabulary developed in connection with order-disorder studies of metallic compounds. This trend is perhaps particularly true of feldspar studies. The suggestion that the various known modifications of alkali feldspar are attributable to ordering actually dates from well before the war. Immediately after the war, as experimental data became available, the notion was extended to the plagioclase feldspars. Synthetic feldspars are similar to those found frequently in volcanic rocks; the forms of alkali feldspar and Ca-poor plagioclases found in plutonic and metamorphic rocks have not yet been successfully synthesized. For a variety of reasons it is now rather generally supposed that volcanic rocks form at temperatures higher than those characteristic of plutonic or metamorphic processes. On the usual assumption that the mineral associations found in common rocks are equilibrium or near-equilibrium assemblages, the forms characteristic of volcanic environments are regarded as stable at "high temperature," whereas those found in the plutonic and metamorphic rocks are considered stable at "low temperature."<sup>1</sup>

In few nonmetallic minerals are the physical differences between the high- and low-temperature forms pronounced, and in many they can be detected only with great

difficulty. Frequently, as in the plagioclase data described below, it is necessary to rely on minor differences in X-ray diffraction patterns. It is now almost a convention to regard the form apparently stable at the higher temperature as disordered and to develop an *ex post facto* argument which makes it seem reasonable that the differences between the X-ray diffraction patterns of the high- and low-temperature forms are such as might occur if the low-temperature form were in some way ordered. There is usually no sound optical theory indicating which (if either) of the patterns is actually characteristic of order (or disorder). Students of the layered minerals, for instance, are fairly well agreed that the appearance of streaks or of weak, poorly resolved additional reflections is indicative of disorder; but in feldspar patterns additional poorly resolved reflections are called subsidiaries, and some workers regard them as indicative of order.

In this latter connection it may be pointed out that, although the  $\delta_c$  subsidiaries of intermediate plagioclase are now often considered evidence of ordering, their displacement from the principal layer lines can be predicted quite nicely from composition on the assumption that the distribution of Al and Si among tetrahedral sites is such as would be expected at complete short-range disorder.

If  $\alpha$  is the proportion of tetrahedral sites occupied by Al (as calculated from the chemical analysis), the expected number of "runs" of Al in any randomly chosen consecutive series of  $N$  such sites is  $N\alpha(1-\alpha)$ , where a run is defined as a sequence of  $i$  Al ions bounded at both ends by an Si ion. Since the entire series contains  $N\alpha$  Al's, the average length,  $E(\bar{i}_{Al})$ , of runs of Al ions is thus  $N\alpha/N\alpha(1-\alpha) = 1/(1-\alpha)$ ; similarly,  $E(\bar{i}_{Si}) = 1/\alpha$ . Figure 1, based entirely on the data of P. Gay, shows the remarkable correlation of the pseudo-periodicity, indicated by the displacement of  $\delta_c$  from the principal layer line, with the average run length of Si at short-range

<sup>1</sup> Although on the whole quite reasonable and conformable with much experimental evidence, this position can sometimes be maintained only by rather dexterous argumentation; in some recent high-pressure base-exchange experiments reported by Wyart, for instance, what many workers now consider the high-temperature forms of feldspar are retained at rather low temperatures but the allegedly low-temperature forms do not survive except at rather high temperatures.

disorder, calculated from the composition.

This is of course not to be construed as proof that the plagioclases studied by Gay are disordered. It is fair to point out, however, that although close dependence of  $\delta_c$  spacing on composition has been known for some time, no argument based on ordering has provided a reasonable explanation of the spacing actually found. To the limits of error of the data, the slope of the solid line shown in figure 1 is unity and its intercept is zero; the average run length of Si at complete disorder and the pseudoperiodicity indicated by the  $\delta_c$  dis-

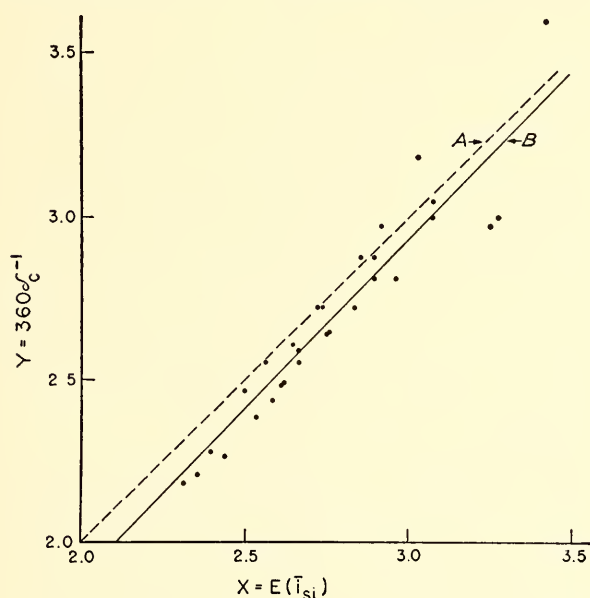


Fig. 1.  $\delta_c$  separations in intermediate plagioclase as a function of Si run length.  $X = E(i_{Si}) = \alpha^{-1}$ ,  $Y = 360 \delta_c^{-1}$ . (A,  $\hat{Y} = X$ ; B, line of best fit calculated from data of P. Gay,  $\hat{Y} = 1.052 X - 0.222$ .)

placement are evidently estimates of the same parent parameter.

#### SHORT- AND LONG-RANGE ORDER

According to now standard definitions, short-range ordering concerns the immediate environment of an atom as judged by the numbers of "right" and "wrong" pairs it forms with its "nearest neighbors," whereas long-range order concerns the emergence and strength of a new over-all periodicity in the crystal. In the ingenious and often rather terrifying "interpenetrat-

ing-lattice" models proposed by statistical thermodynamicists, the elimination of wrong pairs automatically generates what is called long-range order by thermodynamicists and a superlattice by crystallographers. It is not the elimination of wrong pairs as such that generates the long-range order in these models, however, but the mechanism by which they are eliminated. The alleged relation between long- and short-range ordering is not to be regarded as something that emerges from arguments based on interpenetrating-lattice models; it is built into them. Models in which long- and short-range ordering are in general quite independent can be constructed, and in view of current interest in mineralogical order-disorder problems consideration of such models seems eminently worth while.

#### THE LINEAR RUN MODEL OF SHORT-RANGE ORDERING

In the sequence *AABAAABBABBAB* there are seven *A*'s and six *B*'s. Defining a "run" as a sequence of elements of one kind bounded at each end by an element of the other kind (or by the beginning or end of the series), the sequence above contains eight runs, two of length 1, one of length 2, and one of length 3 in *A*; two of length 1 and two of length 2 in *B*.

Run sequences of this type have been studied in considerable detail, perhaps chiefly in connection with statistical quality control, and several of their characteristics useful in a discussion of short-range ordering are now well known. Chief among them is the expected number of runs, from which may be calculated both the average length of run in either element and the numbers of right and wrong pairs at complete disorder or complete short-range order.

Given a sequence of sufficient length, in which the probability that any particular site will be occupied by an *A* is simply the ratio of the number of *A*'s to the number of (*A*'s + *B*'s), the expected number of runs



is  $E(d) \cong 2N\alpha\beta$ , where  $N$  is the number of elements (or sites),  $\alpha = \Sigma(A)/\Sigma(A+B)$ , and  $\beta = 1 - \alpha$ .<sup>1</sup>

Now the probability of occupancy specified above is precisely that which obtains, in theory, at complete disorder. From the definition of a run it is obvious that the last element in each run and the first element of the succeeding run form a right pair, and that there can be no other right pairs. Since the last element of the last run remains unpaired, the expected number of right pairs at complete disorder is thus 1 less than the number of runs, or

$$E(P_{AB}) = E(d) \cong 2N\alpha\beta - 1$$

Coupling each site with its next adjacent site, it is evident that a sequence of  $N$  sites yields a total of  $(N-1)$  pairs, and since  $(2N\alpha\beta - 1)$  of these pairs are right, the remaining  $N(1 - 2\alpha\beta) = N\alpha^2 + N\beta^2$  must be wrong, i.e. must juxtapose two like elements as nearest neighbors. It is easily shown that  $N\alpha^2$  of these are in  $A$ ,  $N\beta^2$  in  $B$ .

At perfect short-range order there are by definition no wrong pairs in the minor element of the sequence, which we shall take as  $A$ . Since right pairs must involve only the first or last element of a run, it follows that at perfect short-range order all runs in  $A$  are of length 1. This means that there will be as many runs in  $A$  as there are  $A$ 's. If boundary corrections are ignored, there will also be as many runs in  $B$  as there are in  $A$ , and so the expected number of runs in the entire sequence will be  $2N\alpha$ . This is again the number of right pairs, for each  $A$  forms a right pair with each of its two bounding  $B$ 's. All the remaining pairs are wrong pairs in  $B$ , and their number is  $N(1 - 2\alpha)$ . These results are shown in table 1. The frequencies of right and

wrong pairs in the two limiting cases are in agreement with those calculated from the interpenetrating-lattice models. Nothing has been said, and except as the quantity  $(\beta - \alpha) \rightarrow 0$  nothing is implied, about long-range order.

TABLE 1. Numbers of Right and Wrong Pairs at Complete Disorder and Complete Short-Range Order

Pair Type	Disorder	Short-Range Order
Wrong: $(AA) \dots\dots$	$N\alpha^2$	0
$(BB) \dots\dots$	$N\beta^2$	$N(1 - 2\alpha)$
Right: $(AB) \dots\dots$	$2N\alpha\beta$	$2N\alpha$

#### DIMENSIONS IN THE RUN MODEL

At complete disorder the run model is essentially independent of direction. It is only necessary that each site occur once and only once, and that in some specified operational sense the  $n$ th site always follow the  $(n-1)$ th and be followed by the  $(n+1)$ th.

In principle, at least, complete short-range disorder ought to be spherically isotropic in a three-dimensional array. As ordering develops, however, its level or intensity varies vectorially, and the lattice of the run sequence must be assigned some direction in the crystal. We may then deal with sets of intersecting run sequences, each site belonging to a member of each set. The members of each set are parallel to each other and intersect members of each other set at a common angle. Since every site is included in one member of each set, and an element of either type may fall on any site, there is still no a priori relation between long- and short-range order. A knowledge of the level of ordering along one set offers no indication of the level of ordering along other sets; in particular, there may be perfect short-range order along one set and complete disorder along all others. (In the run model this could be true of long-range ordering as well.)

<sup>1</sup> From the definition we may also write

$$E(d) \cong 2 \frac{\Sigma(A)\Sigma(B)}{\Sigma(A+B)}$$

Although this and all succeeding estimates are large-sample approximations, the approximations are very good even for  $N$  much smaller than is likely to be required in crystal chemistry.

#### THE NUMBERS AND LOCATIONS OF NEAREST NEIGHBORS

The interpenetrating-lattice models used in the standard treatments are so constructed that no site may have as nearest neighbor another site on the same sublattice. In the run model, on the contrary, the nearest neighbors of the  $n$ th site are always the  $(n-1)$ th and  $(n+1)$ th sites of the same run sequence. Thus if a site is a member of only one run sequence it has only 2 nearest neighbors. In a plane net a site may be a member of two or three intersecting run sequences and may accordingly have 4 or 6 nearest neighbors. In three dimensions any site may be a member of three or four intersecting run sequences, and thus may have 6 or 8 nearest neighbors. The interpenetrating-lattice models are not so limited or inflexible with regard to the number of nearest neighbors, a parameter of considerable importance in thermodynamic calculations.

#### SOME PRELIMINARY EXPERIMENTAL RESULTS

*Instrumentation.* Enough has been said to indicate that knowledge of the influence of disorder on diffraction effects would be of considerable value in studies of solid-state transitions. Direct mathematical analysis is rather forbidding, and at present there seems no other approach to the problem in three dimensions. To produce two-dimensional models (or masks), however, is comparatively simple, and the diffraction patterns of such masks are readily observed in an optical diffractometer of the kind first described by Taylor, Hughes, and Lipson. In this apparatus the optical train consists of a point source, filters, a collimating lens, a collecting lens, and a microscope or camera. The mask is placed in the collimated beam (between collimator and collector), and its (Fraunhofer) diffraction pattern is observed or photographed at the focal plane of the collector. During the last two months of the report year a small instrument of this type was constructed, with a 2-watt concentrated arc light as point source. The lenses are of 1-inch

working diameter, and their focal lengths are such that the image is brought to final focus less than 2 feet from the point source. Its dimensions make the instrument both inexpensive to build and convenient to use. The relatively short focal lengths of the collimator and collector generate a rather small final diffraction pattern, a limitation not critical for present purposes. The narrowness of the collimated beam is a more serious handicap, for it requires the use of rather small masks.

For the production of suitable masks a procedure combining punching and photographic reduction has been developed. A more complete description of the instrument and an account of the procedure for making masks is being prepared for publication elsewhere. To date only masks containing a single "atom"—i.e. in which all openings are of the same diameter—have been prepared, and emphasis has been placed on the effect of "mistakes" in the stacking of layered structures. Layering is particularly useful as a starting point, not only because masks portraying varying levels of ordering are easily prepared but also because it has been examined theoretically.

*Equal numbers of layers in each of two positions.* This is the case on which A. J. C. Wilson builds his theory of the optical effect of mistakes in layered structures. The  $a$  and  $b$  axes lie in the plane of layering, the  $c$  axis normal to this plane, all the layers are supposed identical, and the mistake consists of a shift of the layer by half the  $b$  axis. The diffraction mask modeling this situation would be a  $bc$  section, with lines containing equal numbers of equally spaced holes in the  $b$  direction and a randomly distributed offset of  $b/2$  from one layer to the next along  $c$ . Figure 2*a* shows such a mask, and figure 2*b* its diffraction pattern.<sup>1</sup> The diffuse streaks cutting the principal layer lines are in accord with the reciprocal lattice calculated by Wilson; the

<sup>1</sup> Figures 2 and 3 are on plate 1. Plates are collected, facing page 192.



weak but clearly resolved reflection midway between layer lines occurs in a region in which his theory evidently indicates no diffraction, either diffuse or resolved. In a mask in which short-range ordering was perfect—that is, one in which there was an offset between each pair of layers—this central spot would be of the same intensity as the principal reflections and there would be no diffuse scatter crossing the layer lines.

The contrast between the observed and expected effect of disorder on diffraction is so striking as to require further study, and this is now in progress. Since the transform of the perfectly ordered array would have a strong central spot, it is tempting to suppose that the presence of a weak spot in this position in the transform of the disordered array merely signals the presence of an undue concentration of *ABAB* . . . sequences in the mask. There is excellent reason to suppose, however, that this is not the correct explanation in this particular case.

*Preponderance of layers in one position.* In the standard derivation, based on Wilson's analysis, the number of layers in each position is, at least by implication, the same. The remaining work to be reported on is concerned with masks in which there is a preponderance of layers in one of the two positions. Crystallographers have evidently not considered this possibility in connection with order-disorder diffraction effects, but even the preliminary results now available suggest that it might be worth close study.

Figure 3 is the diffraction pattern of a mask containing a rigid succession of two

layers in one position followed by one in the other, e.g. *BBABBABBA*. . . . This arrangement is characterized by perfect short- and long-range order; e.g., all *A*'s occur in runs of length 1 along *c*, and the "repeat distance" along *c* is 3 layers. There are four principal reflections at the corners of a square, an exceedingly weak one between each pair of principals on the layer lines, and two bright ones symmetrically spaced at  $\frac{1}{3}$  and  $\frac{2}{3}$  of the distance between layer lines.

Figure 4a<sup>2</sup> is a 2:1 (actually 35 per cent) mask in which there is perfect short-range but no long-range order, and figure 4b is its diffraction pattern. The principals and weak subsidiaries are as in figure 3a, but instead of two bright reflections at  $\frac{1}{3}$  and  $\frac{2}{3}$  of the layer line interval there is a single, evidently composite, large one at  $\frac{1}{2}$  that interval.

Figure 5a is a 35 per cent mask in which there is complete disorder—i.e. the probability that any layer is an *A* is simply the proportion of *A*'s in the parent population of layers. Figure 5b is the diffraction pattern of this mask. The four principals are as in figures 3 and 4, but there is much diffuse scatter, and there are also two well resolved subsidiaries at  $\frac{1}{6}$  and  $\frac{5}{6}$  the layer line interval.

The patterns yielded by perfect long- (and short-) range order, perfect short-range order, and complete disorder are thus clearly differentiable. Investigation of the nature of the passage from one pattern to either of the others, as well as the study of similar diffraction effects for different *A:B* ratios, is now in process.

<sup>2</sup> Figures 4 and 5 are on plate 2.

## BASALT MAGMAS

*H. S. Yoder, Jr., and C. E. Tilley*

Each of the two major basalt magma types, the tholeiitic basalt type and the alkali basalt type, has been nominated as the parental or primitive magma. Some believe that a third magma as yet unobserved is in fact the primary magma, and

others view the various magmas as unrelated, each giving rise to its own rock types independently.

New experimental data bear on the problem of the alleged primary magma. Specimens of natural basalt representative of

three magma types, (a) tholeiite, (b) alkali basalt, and (c) high-alumina basalt, were selected for thermal study at atmospheric pressure. Types (a) and (b), represented by specimens from Hawaii, conform to the two chief magma types recognized by the authors of the Mull Memoir, and (c), represented by a nonporphyritic Warner basalt from California, is chemically comparable to the Porphyritic Central type of Mull. The chilled marginal facies of the Skaergaard layered gabbro of East Greenland conforms closely to this type (Tilley, 1950). Analyses of four rocks of the three types are given in table 2, and the norms in table 3.

The first two analyses are of tholeiitic type: (1) the 1921 Kilauea lava, an olivine-enriched example discussed in last year's report; and (2) a prehistoric lava from Kilauea, a typical silica-saturated basalt corresponding more closely to the primitive Kilauean liquid (Powers, 1955).

The pyroxenes of the four rocks have been isolated and analyzed; the results of the analyses are expressed in terms of the principal pyroxene components in figure 6. Three fractions of pyroxene (A, B, C) obtained from the 1921 lava and two (D, E) from the prehistoric lava provide evidence that some fractionation took place during the crystallization.

The pyroxenes have compositions typical of those from the three magma types. In general, the pyroxenes of the alkali basalts are along the Di-Hd join, while the tholeiitic pyroxenes are more pigeonitic.

Each of these basalts, as well as others, was held at various temperatures through the courtesy of J. F. Schairer. Each basalt is a single bulk composition in a multicomponent system, and its crystallization history can be determined in the usual way by the quenching method. Small samples were dried at 110° C, sealed in platinum tubes, and held for 1 to 24 hours at constant temperature and atmospheric pressure. Because of loss of iron to the platinum container and some oxidation of the iron, the temperatures stated should be considered

approximate. The results are given in figure 7.

For the 1921 flow of Kilauea, the liquidus is at 1235° C, and olivine is the first major phase to appear. At about 1190° C pyroxene comes in, and at 1170° C these are joined by plagioclase. At 1090° C the charge is all crystalline. The temperatures

TABLE 2.—Chemical Analyses of Basalts

	1	2	3	4
SiO <sub>2</sub> .....	49.16	51.18	48.27	49.28
Al <sub>2</sub> O <sub>3</sub> .....	13.33	14.07	18.28	15.98
Fe <sub>2</sub> O <sub>3</sub> .....	1.31	1.35	1.04	4.11
FeO .....	9.71	9.78	8.31	7.94
MnO .....	0.16	0.17	0.17	0.19
MgO .....	10.41	7.78	8.96	4.44
CaO .....	10.93	10.83	11.32	9.55
Na <sub>2</sub> O .....	2.15	2.39	2.80	3.47
K <sub>2</sub> O .....	0.51	0.44	0.14	1.26
H <sub>2</sub> O <sup>+</sup> .....	0.04	0.10	0.15	0.39
H <sub>2</sub> O <sup>-</sup> .....	0.05	0.01	0.07	0.53
P <sub>2</sub> O <sub>5</sub> .....	0.16	0.15	0.07	0.23
TiO <sub>2</sub> .....	2.29	2.10	0.89	3.06
Cr <sub>2</sub> O <sub>3</sub> .....	0.09	0.05	....	0.02
Total .....	100.30	100.40	100.47	100.45

- 1. Tholeiite basalt: 1921 lava, Kilauea caldera, Hawaii, No. 57364 (Cambridge University Collection). Analyst: J. H. Scoon.
- 2. Tholeiite basalt: prehistoric flow, National Park Quarry on highway 0.75 mile NE of Volcano Observatory, Kilauea No. 57358 (Cambridge University Collection). Analyst: J. H. Scoon.
- 3. High-alumina basalt: Warner flow, 4 miles SE of East Sand Butte, Medicine Lake Highlands, Calif., No. 127-ML-295. Collected by C. A. Anderson. Analyst: J. H. Scoon.
- 4. Alkali basalt: Papalele Gulch, near highway NE of Mauna Kea, Hawaii, No. 60464 (Cambridge University Collection). Analyst: J. H. Scoon.

observed by Jagger at the Halemaumau crater in 1921 were 1190° and 1200° C. The above results are in accord with his observations and suggest that the water pressure of the magma was very low. The most significant observation, however, is that all three major phases appear within 65° C. Dip samples of the lava in 1911 showed all three phases present even though the crystal content was only a few



per cent. The index of refraction of the glass so obtained was given as 1.605 by Merwin. The glass prepared in the laboratory by completely melting the 1921 flow basalt had an index of refraction of 1.608. Note also that in the laboratory the basalt had a small interval of crystallization of about 150° C. A prehistoric lava from Kilauea (fig. 7), which has less olivine, shows approximately the same results but has a liquidus at 1195° C. All three major phases appear within a small range of temperature, 40° C. The total range of crystallization is only 130° C. The liquidus for the 1887 basalt from Mauna Loa is about the same temperature as the Kilauean basalts, each producing olivine as the first major phase. The alkali basalt from Mauna Kea has a liquidus of 1185° C, and plagioclase is the first major phase to

appear. Olivine and pyroxene come in together at about 1160° C, and at 1040° C the charge is all crystalline. Again, a narrow range of temperature in which all three major phases appear, 25° C, is observed. The total range of crystallization is only 150° C. A representative of the third major basalt type is the high-alumina basalt from Medicine Lake Highlands, California. This basalt yields approximately the same results.

The significant conclusions from this cursory study of representatives of the three major basalt types are: (1) all three major phases appear in a short interval of temperature under the experimental conditions; (2) all three major phases appear

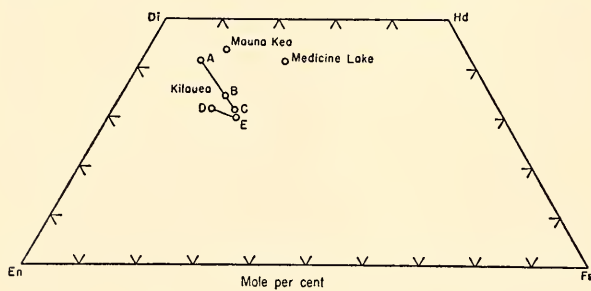


Fig. 6. Plot of pyroxenes from rocks of table 2 in the system diopside (Di)-hedenbergite (Hd)-enstatite (En)-ferrosilite (Fs). The fractions A, B, and C are from the 1921 flow, and D and E from a prehistoric flow, of Kilauea.

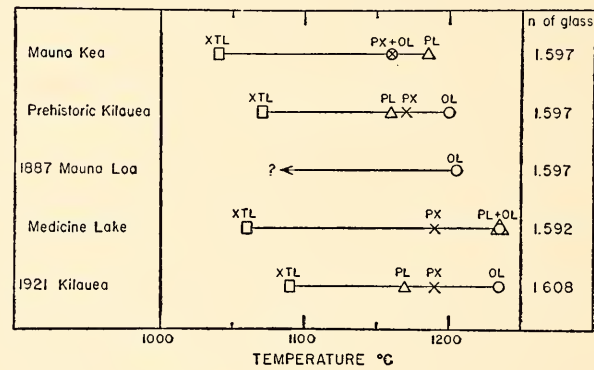


Fig. 7. Results of thermal treatment of selected basalts representative of major basalt magma types. The abbreviations are for olivine (OL), plagioclase (PL), pyroxene (PX), and all crystalline (XTL).

TABLE 3. Norms of Basalts Given in Table 2

	Tholeiite 1921 Kilauea	Tholeiite Prehistoric Kilauea	High-Alumina Basalt Medicine Lake	Alkali Basalt Mauna Kea
Qz .....	....	0.30	....	....
Or .....	2.78	2.22	0.56	7.50
Ab .....	17.82	20.96	23.58	29.34
An .....	25.30	26.13	36.97	24.32
Di .....	22.93	22.04	15.23	17.97
Hy .....	15.35	22.44	....	6.43
Ol .....	9.14	....	20.55	1.83
Mt .....	2.09	1.86	1.39	6.03
Il .....	4.41	3.95	1.67	5.78
Ap .....	0.34	0.34	0.17	0.34
H <sub>2</sub> O <sup>±</sup> .....	0.09	0.11	0.22	0.92
Total .....	100.25	100.35	100.34	100.46

together at about the same temperature ( $1160^{\circ}$  to  $1170^{\circ}$  C), irrespective of the bulk composition of the basalt; (3) the total range of crystallization is small, of the order of  $150^{\circ}$  C; (4) olivine or plagioclase appears on the liquidus to the exclusion of pyroxene for the basalts studied. Powers (1955) records the fact that no augite phenocrysts are found in most of the lavas of the shield-building stage at Hawaii.

These data can be interpreted in terms of the synthetic system of simplified basalts as determined in the laboratory. To repre-

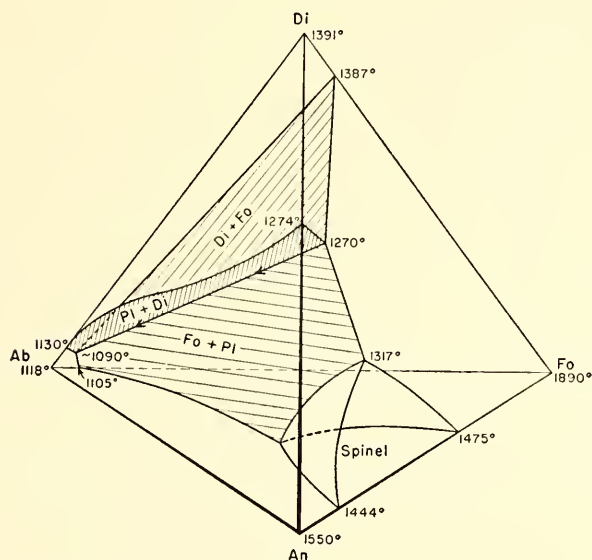


Fig. 8. The "simplified basalt" system diopside (Di)-albite (Ab)-anorthite (An)-forsterite (Fo) based on the experimental studies of Osborn and Tait (1952), Bowen (1915), and Schairer (unpublished data). Certain liberties were taken near the corner Ab, which are amplified in part by figure 9.

sent the compositions of a simple basalt (pyroxene + olivine + plagioclase), the tetrahedron diopside (Di)-forsterite (Fo)-albite (Ab)-anorthite (An) was constructed from the studies of Osborn and Tait (Di-Fo-An), Bowen (Di-Ab-An), and Schairer (unpublished data: Fo-Ab, Di-Ab). The system given schematically in figure 8 is, of course, only pseudoquaternary, since the compositions of all the phases cannot be represented by the components chosen. If the spinel field is neglected, there are three volumes, each representing a major phase—diopside, forster-

ite, and plagioclase. Two phases are in equilibrium with liquid along the shared surfaces, and all three phases are in equilibrium with liquid along the one common curve, the four-phase curve. In the heating experiments described, all three phases appeared over a small range of temperature. For this reason the composition of the basalts must lie very close to a similar four-phase curve. Whether olivine or plagioclase (or even pyroxene) precipitates out first is of small importance. What is important is the temperature at which all three phases begin crystallizing together. Each composition should on cooling reach the four-phase curve at a characteristic temperature. Liquids at higher temperatures on the four-phase curve can give rise, through fractionation, to liquids at a lower temperature on the curve. Liquids at the lower temperatures cannot yield a magma having a higher temperature on the four-phase curve. From the data on the natural rocks given in figure 7, it is seen that neither the alkali basalt nor the tholeiite basalt can be specified as the parent, since they reach the "four-phase curve" at about the same temperature. On the basis of the data presented, it may be tentatively concluded that all these basalts are themselves a product of the same melting process, the diversity arising as a result of different initial bulk compositions.

If the simple basalts represented in figure 8 fractionated, the liquid would descend to the point marked  $\sim 1090^{\circ}$  C. Certain liberties were taken to simplify the relations near the Ab corner, and now these must be examined more closely. Schairer has restudied the join Ab-Di in the larger aspect of the nepheline-silica-diopside join, which is not ternary (fig. 9). Here enters another difficulty in choosing the alleged parental basalt. Consider a bulk composition in the diopside field on the Ab-Di join. The liquid stays in the plane until it hits the plagioclase field boundary curve. Then, if the liquid were slightly saturated in silica, its fractionated liquids would trend toward silica. If the liquid were



slightly undersaturated, it would go to the nepheline side. In examining the original liquid, very careful analytical work would be required to determine in advance which way it would go. A similar situation arises when compositions near the Ab-An join in the nepheline-silica-anorthite system (Schaier, unpublished) are considered. In all the systems involving potash, such as An-leucite-silica (Schaier and Bowen),

of the two major magma types, and its behavior bears on their relationships. The tholeiite magmas characteristically show the reaction relation with olivine; the alkali basalt magmas do not. These observations can be interpreted in terms of Bowen's diagram for Di-Fo-silica (fig. 10). The tholeiites must start their crystallization in the olivine field in the area formed by a line joining  $\text{Mg}_2\text{SiO}_4$  to the point where

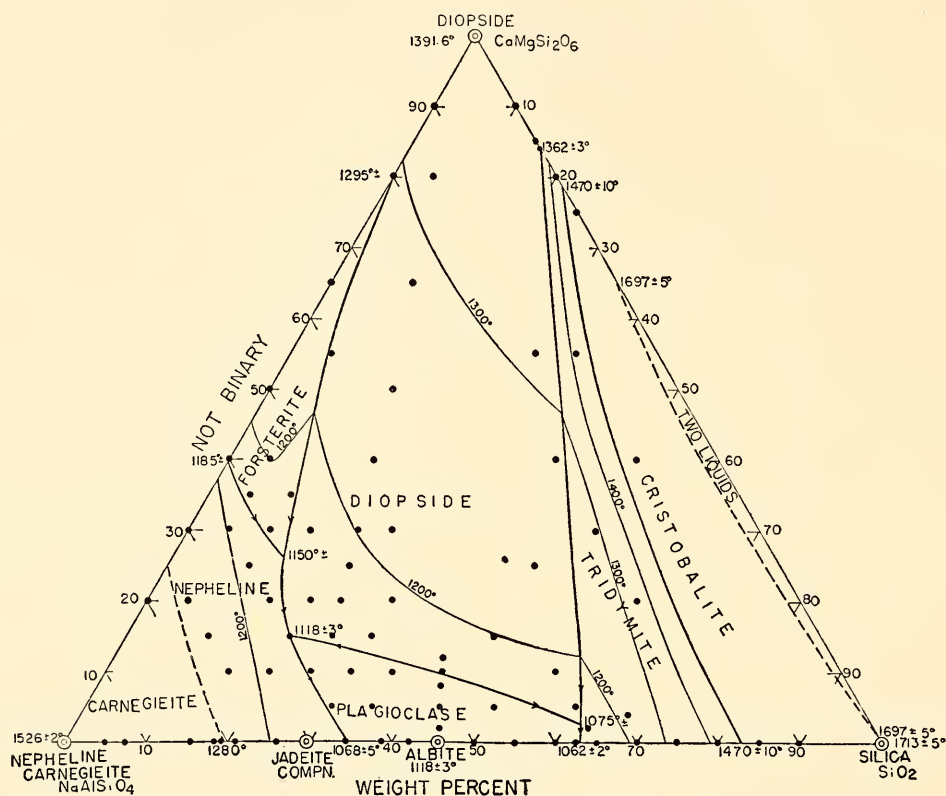


Fig. 9. The system  $\text{CaMgSi}_2\text{O}_6$ - $\text{NaAlSi}_3\text{O}_8$ - $\text{SiO}_2$  (Schaier, unpublished data given in Yoder, 1950, with permission). The join albite-diopside, which is not binary, is not shown.

Di-leucite-silica (Schaier and Bowen), Fo-leucite-silica (Schaier), and fayalite-leucite-silica (Bowen and Schaier), the liquids trend toward silica and not toward the feldspathoid. It is clear from these considerations that no one magma can go to both sides if only the simple major phases are considered. On these grounds it must be concluded that a single magma cannot produce both a tholeiite trend and an alkali trend by fractionation. The differences between the magmas capable of producing such trends, however, may be so small as to be not readily detectable.

Olivine plays an interesting role in each

the pyroxene boundary curve crosses the  $\text{MgSiO}_3$ - $\text{CaMgSi}_2\text{O}_6$  join and the pyroxene boundary curve. The alkali basalts apparently begin their crystallization in the remaining area of the olivine field, or in the pyroxene field near the diopside corner to the left of the  $\text{MgSiO}_3$ - $\text{CaMgSi}_2\text{O}_6$  join. To support this view, Powers notes that the lavas showing augite phenocrysts are usually assigned to the declining stage of activity in the Hawaiian volcanoes, the alkali-rich stage.

Water must be considered among the agents effective in altering the course of crystallization, and the oxidation or reduc-

tion of iron may account for alternative courses. The problem of the alleged parental basalt seems to hinge on finding suitable mechanisms to bridge the apparent

barrier between the saturated and unsaturated magma types. The effects of water as a possible mechanism are now under study.

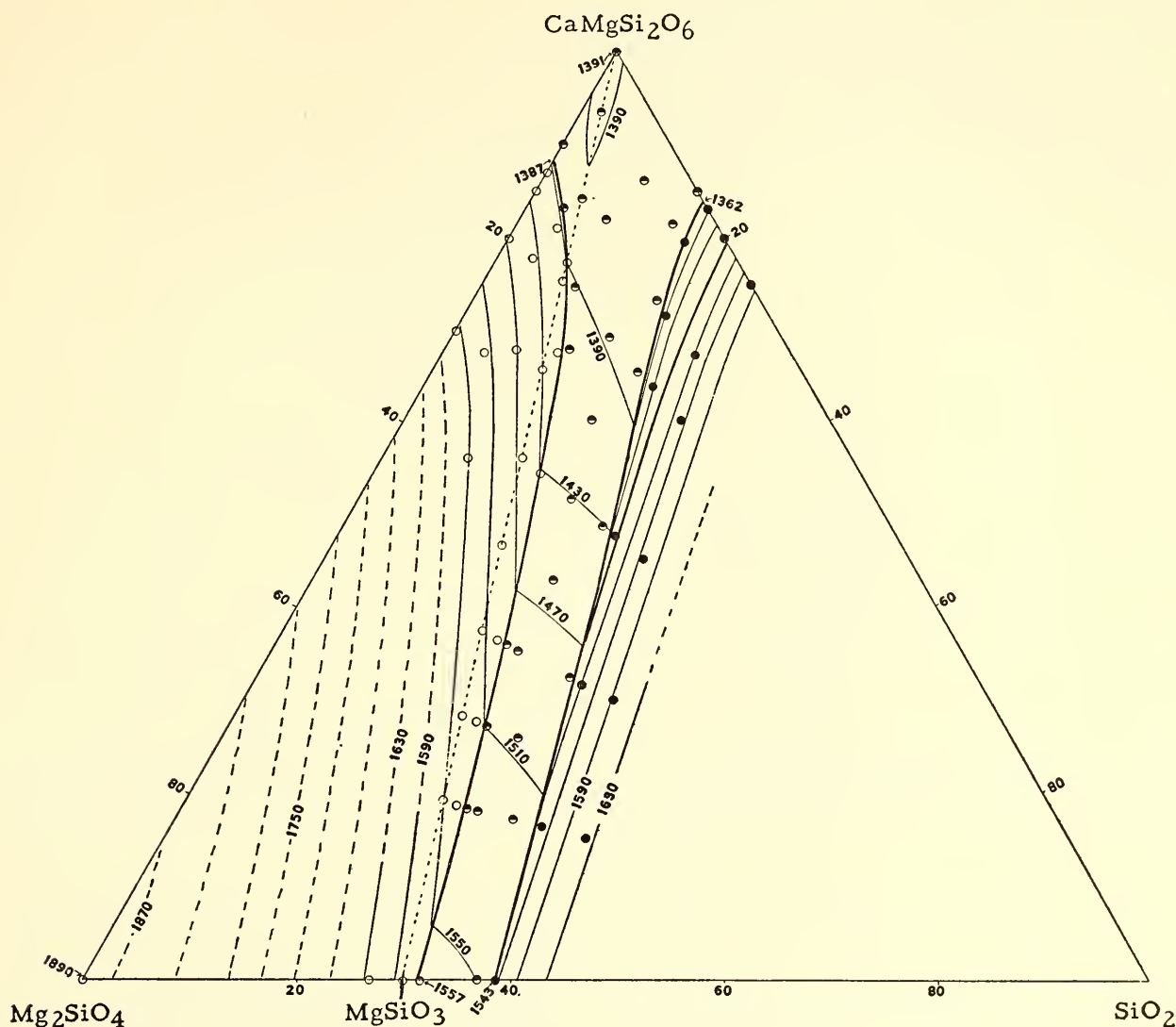


Fig. 10. The system  $\text{CaMgSi}_2\text{O}_6$ - $\text{Mg}_2\text{SiO}_4$ - $\text{SiO}_2$  (Bowen, 1914). The join  $\text{CaMgSi}_2\text{O}_6$ - $\text{MgSiO}_3$ , which is not binary, is dashed.

## STABILITY OF ANNITE

*H. P. Eugster*

Preliminary data on the stability of the ferrous biotite annite were reported last year. Since then experimental work on this mineral has been completed up to 2000 bars total pressure. The revised equilibrium diagrams are presented in figures 11, 12, 13, and 14. Of the three independent variables, temperature  $T$ , total pressure  $P_{\text{tot}}$  ( $=P_{\text{H}_2\text{O}}$ ), and partial pressure of oxygen  $P_{\text{O}_2}$ , only two, namely  $T$  and  $P_{\text{tot}}$ , can be varied continuously.  $P_{\text{O}_2}$  is defined

by the five buffers that surround the semi-permeable sealed platinum tube. The buffers are mixtures of iron oxides, fayalite, and quartz. Hydrogen formed by the dissociation of water and passing through the platinum tube acts as a transfer agent in equalizing  $P_{\text{O}_2}$  between buffer and sample.  $P_{\text{H}_2}$  and  $P_{\text{O}_2}$  are not independent variables, since the dissociation of water is constant for a given  $T$  and  $P_{\text{tot}}$ . The five buffers used are (a) fayalite + iron + quartz,



(b) iron + wüstite, (c) wüstite + magnetite, (d) magnetite + quartz + fayalite, and (e) hematite + magnetite.

The  $P_{O_2}$ - $T$  curves for these pairs are

presented in figures 11 and 12. In terms of the three variables  $T$ ,  $P_{tot}$ , and  $P_{O_2}$ , annite occupies a volume within which it represents the stable phase. This volume

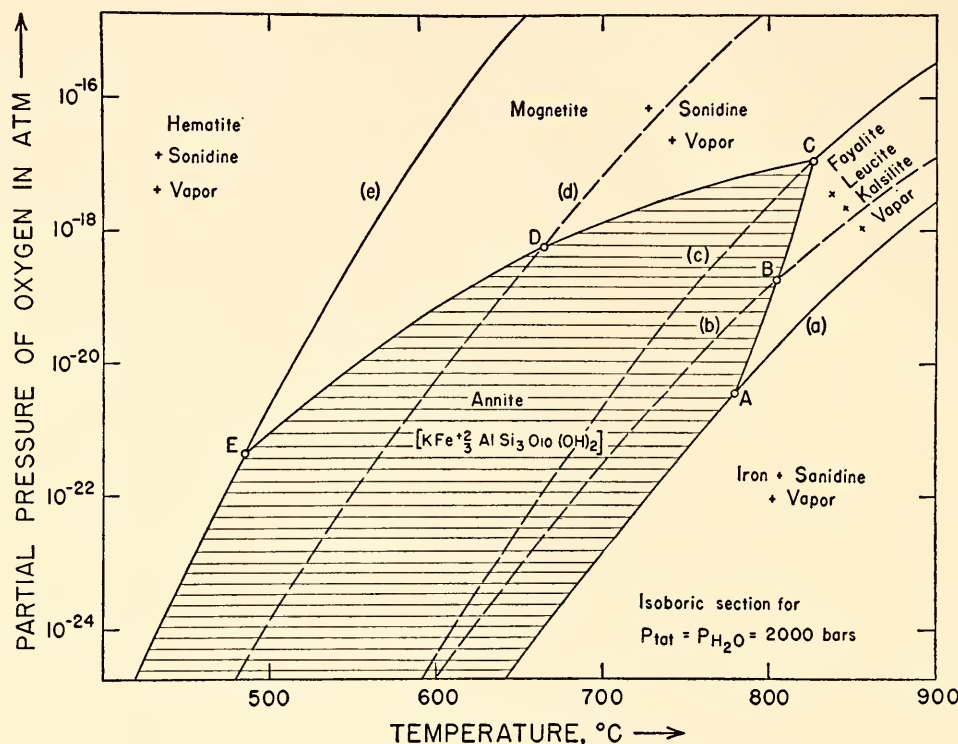


Fig. 11. Isobaric section of the stability field of annite for  $P_{tot}=P_{H_2O}=2000$  bars. The curves are  $P_{O_2}$ - $T$  curves for the following buffers: (a) iron + fayalite + silica, (b) iron + wüstite, (c) wüstite + magnetite, (d) fayalite + magnetite + silica, and (e) magnetite + hematite. For further explanation see text.

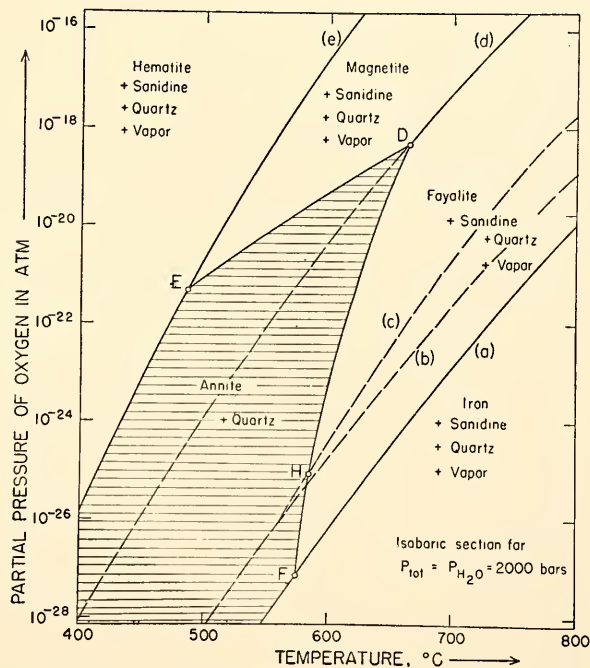


Fig. 12. Isobaric section of the stability field of annite + quartz for  $P_{tot}=P_{H_2O}=2000$  bars. For the significance of curves (a), (b), (c), (d), and (e) see legend to figure 11 and text.

is separated by curved surfaces from four other volumes representing the phase assemblages hematite + sanidine + vapor, magnetite + sanidine + vapor, fayalite + leucite + kalsilite + vapor, and iron + sanidine + vapor. Figure 11 shows a section through these volumes at  $P_{tot}=P_{H_2O}=2000$  bars. The effect of  $P_{O_2}$  and  $T$  on the location of the reversible equilibria is clearly discernible. For points A, B, C, D, and E, reversibility of the reactions has been demonstrated and the temperatures are known within  $\pm 5^\circ$  C. Figure 12 shows a section also at  $P_{tot}=P_{H_2O}=2000$  bars, but for the bulk composition of annite + quartz. Fayalite + sanidine + quartz can coexist over a rather wide  $P_{O_2}$ - $T$  field, thereby restricting the stability of annite in the presence of quartz considerably.

Figure 13 shows  $P_{tot}$ - $T$  curves for the reactions studied to demonstrate the influ-

ence of total pressure. Along the univariant curves,  $P_{O_2}$  is not constant but changes according to the  $P_{O_2}$ - $T$  curves of the buffer assemblages used for a specific reaction. The  $P_{tot}$ - $T$  curves show slopes common to curves of most hydration-dehydration reactions.

Figure 14 is a three-dimensional drawing showing the stability volumes of the five phase assemblages for the annite bulk

which annite represents the stable phase. It is convenient to present the stability relations of hydrous iron silicates in terms of total pressure, partial pressure of oxygen, and temperature as independent variables. But it should be remembered that a representation in terms of  $P_{tot}$ ,  $P_{H_2}$ , and  $T$  is equally justified. This is particularly significant, since hydrogen and not oxygen equalizes gradients between buffer and

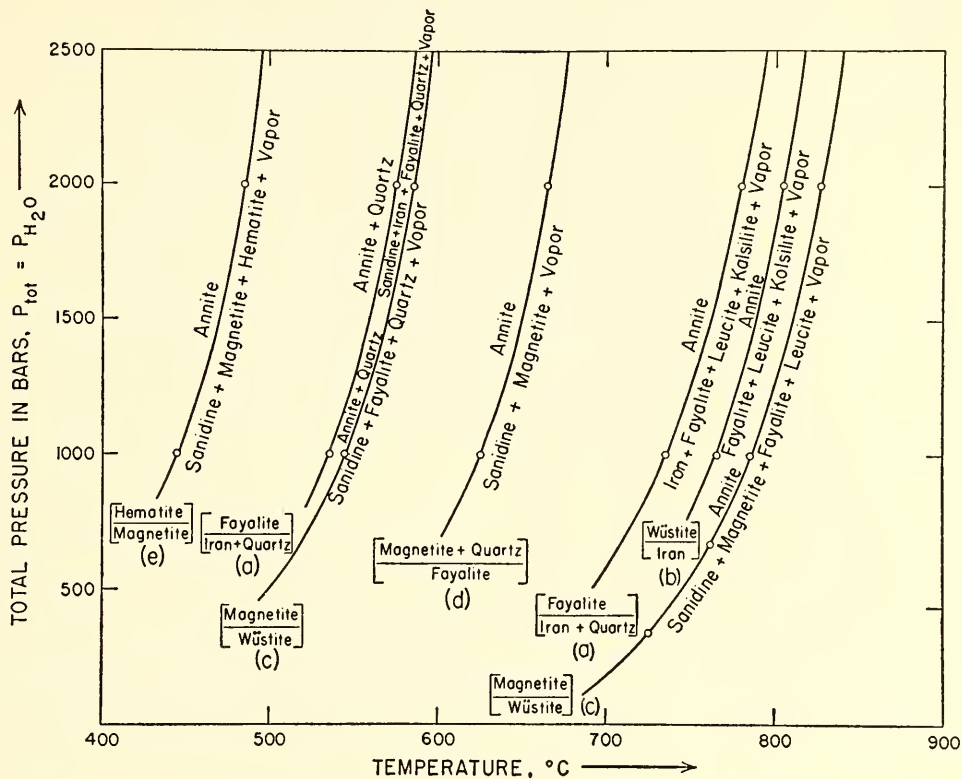


Fig. 13. Temperature-total pressure ( $P_{tot}=P_{H_2O}$ ) diagram for systems annite and annite+quartz. For each of the selected univariant curves the partial pressure of oxygen is equal to that of the buffer used (brackets) and changes with temperature accordingly.

composition. Two additional volumes are not represented, because their existence could not be verified experimentally. From phase-rule considerations we know that the magnetite+sanidine+vapor volume must be separated from the fayalite+leucite+kalsilite+vapor volume by a narrow volume for magnetite+fayalite+leucite+vapor, whose width is narrower than the experimental error ( $\pm 5^\circ C$ ). A second narrow volume for iron+fayalite+leucite+vapor must separate the volumes for fayalite+leucite+kalsilite+vapor and for iron+sanidine+vapor. These modifications do not affect the volume within

sample. In the geologically important region the partial pressures of hydrogen range from tenths to several hundred bars.

Annite is the first hydrous phase for which the relationships in a portion of the  $P_{tot}$ - $P_{O_2}$ - $T$  space have been worked out. The reactions involved are hydration-dehydrations combined with reduction-oxidations. The two main conclusions derived from the work on annite can be stated as follows: Iron silicates possess a definite range of partial pressures of oxygen over which they are stable. This range changes with change in temperature. In the case of annite the upper limit for  $P_{O_2}$



is that of the magnetite-hematite boundary. The equilibrium temperatures of all reactions that show a transfer of hydrogen or

oxygen between some of the participating phases will depend on the magnitude of the partial pressure of oxygen.

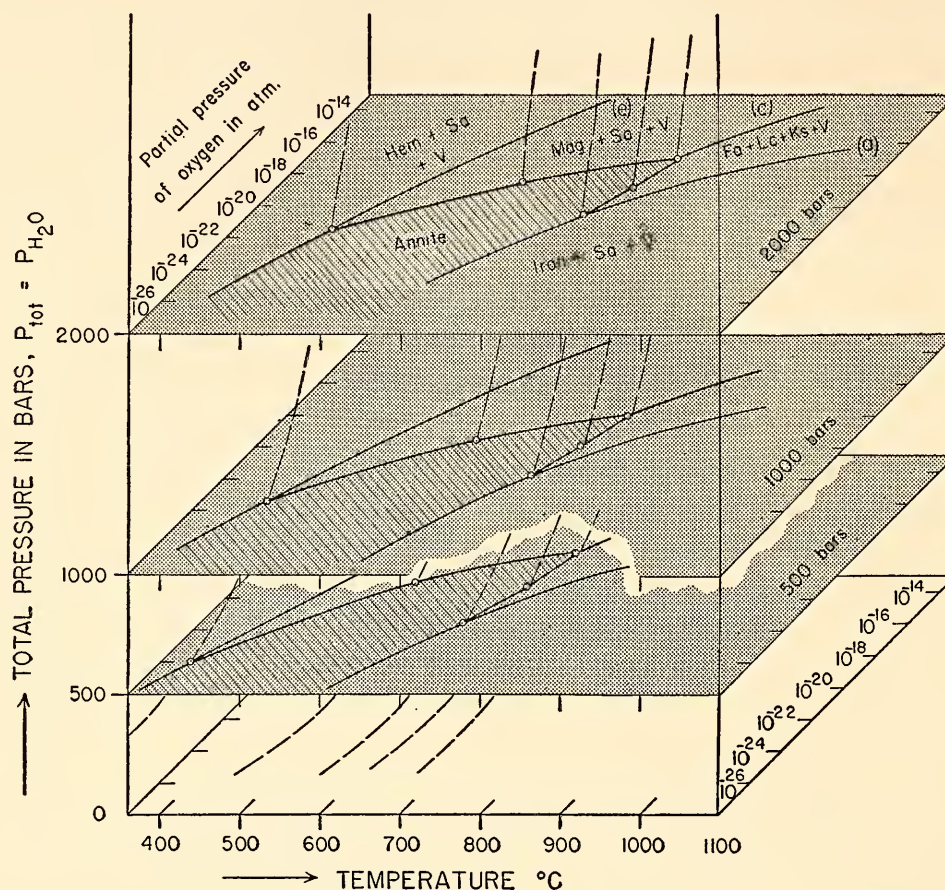


Fig. 14.  $P_{\text{tot}}-P_{\text{O}_2}-T$  model of the stability volume of annite, presented in isobaric sections. The curved surfaces separating the five individual volume are defined in this drawing by  $P_{\text{O}_2}-T$  and  $P_{\text{tot}}-T$  curves.

## THE AGE OF ROCKS AND MINERALS

*(A co-operative program of the Geophysical Laboratory and the Department of Terrestrial Magnetism of the Carnegie Institution of Washington)*

G. L. Davis, G. R. Tilton, L. T. Aldrich,<sup>1</sup> G. W. Wetherill,<sup>1</sup> and H. Faul<sup>2</sup>

We have to remember that while nature is complex with time-less subtlety, human thought issues from the simple-mindedness of beings whose active life is less than half a century.—A. N. Whitehead, 1919

Now that reliable methods of age determination have been developed a number of additional problems have become accessible to study. Fossils have been used to establish relative ages in post-Precambrian times, but they can give only a rough indication of the actual periods of time

involved. The fossil time scale currently employed is based on only four points, all of questionable value, either because of lack of concordancy in the isotopic ages used in calibration or because the stratigraphic position of the samples cannot be accurately established. The recently developed ability to measure ages on granite greatly increases the number of samples suitable for gaining information for this time scale.

In Precambrian rocks, fossils are absent, and relative ages are difficult to establish over any great distances in the field.

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Younger orogenic belts, such as the Appalachian Province, contain considerable bodies of igneous and metamorphic rocks that were formed at about the same time. Perhaps, when a sufficient number of age measurements are completed in the Precambrian, vestiges of a number of older orogenic belts will be found. For example, the wide prevalence of 1000-million-year-old igneous rocks in the Grenville is generally believed to indicate such a belt. A major long-range goal of work on age determination is to establish the space-time relationship of orogenic chains of the past as a prelude to understanding the role played by such belts in the development of the continents.

Reliable age determinations depend on finding concordant ages—that is, agreement between two uranium-lead ages and between uranium-lead, rubidium-strontium, and potassium-argon ages. Such agreement is not always found. Often the rubidium-strontium and potassium-argon ages agree with each other but the two uranium-lead ages are discordant. Sometimes even the rubidium-strontium and potassium-argon ages are discordant. Potentially, useful information is buried in these results. It may be possible in the future to specify within reasonably narrow limits the conditions that brought about the discordancies and thereby gain information on the post-crystallization history of these rocks. Workers in the field of age determination have not yet begun a serious attack on this problem.

In the past year some progress has been made in all the phases of activity mentioned. Precambrian problems have been studied in the Rocky Mountains of western United States and at Sudbury, Ontario. Information of potential use to the fossil time scale has been obtained from the Hercynian Chain in western Europe and from the Wichita Mountains in Oklahoma. A study was begun to test the influence, if any, of orogenies in producing discordant ages in the mineral zircon. These results will be discussed in more detail below.

#### THE DECAY CONSTANTS OF $\text{Rb}^{87}$ AND $\text{K}^{40}$

A year ago the report of this group stated that the uncertainties in the decay constants of  $\text{Rb}^{87}$  and  $\text{K}^{40}$  appeared to be resolved by comparing  $\text{K}^{40}\text{-A}^{40}$  and  $\text{Rb}^{87}\text{-Sr}^{87}$  ratios in micas with the concordant  $\text{U}^{238}\text{-Pb}^{206}$  and  $\text{U}^{235}\text{-Pb}^{207}$  ages given by uraninites from the same mineral assemblages. Since the decay constants of  $\text{U}^{238}$  and  $\text{U}^{235}$  are known to within 2 to 3 per cent, it was possible to calculate the decay constants for  $\text{Rb}^{87}$  and  $\text{K}^{40}$  from the uranium-lead age. The calculated constants were in agreement for six mica-uraninite assemblages ranging in age from 370 to 2700 million years. This agreement led to the belief that alteration of the Rb-Sr and K-A ratios in the micas by processes other than radioactive decay was not a serious problem. Crystal counting experiments on  $\text{K}^{40}$  by Wetherill (see Report of the Director of the Department of Terrestrial Magnetism) during the past year have confirmed the geologic value of  $\lambda_e$  for the decay of  $\text{K}^{40}$  to  $\text{A}^{40}$  within 5 per cent, assuming that none of the  $\text{K}^{40}$  decays directly to the ground state of  $\text{A}^{40}$ . Wetherill's value for  $\lambda_e$  is enough higher than the geologic value to indicate that even the best micas may have lost on the average 4 or 5 per cent of the  $\text{A}^{40}$ . The geologically determined constants are used for purposes of age determination, but these constants must agree closely with the laboratory values if any great confidence is to be placed in age measurements using them. The agreement now appears to be close for most micas.

#### PROBLEMS IN THE PRECAMBRIAN

*The occurrence of 1300- to 1400-million-year-old granitic rocks in western United States.* Rubidium-strontium and potassium-argon ages have been measured on eleven micas, including biotite, muscovite, and lepidolite, from Precambrian granites and pegmatites occurring in the Cordilleran System in Arizona, New Mexico, Colorado, and Wyoming. The ages are given in table 4. All these micas have ages



between 1300 and 1400 million years, indicating that there was a widespread crystallization of granitic rocks at this time and that the micas have preserved their ages in spite of more recent events, including the Laramide orogeny. These mica ages are believed to date a period of regional rock formation of a type comparable to the later periods of igneous intrusion and

TABLE 4. Age Determinations on Micas from Western United States

K-A ages are calculated from decay constants of  $K^{40}$  of  $\lambda_e=0.557 \times 10^{-10} \text{ yr}^{-1}$ ,  $\lambda_\beta=4.72 \times 10^{-10} \text{ yr}^{-1}$ , or a total half-life for  $K^{40}$  of  $1.31 \times 10^9 \text{ yr}$ . Rb-Sr ages are calculated using a half-life for  $Rb^{87}$  of  $50 \times 10^9 \text{ yr}$ .

Location	Age, million years	
	K-A	Rb-Sr
1. Gneiss, Zoroaster Creek, Grand Canyon, Ariz.....	1380	1350
2a. Lawler Peak granite, Bagdad, Ariz. ....	1410	1390
2b. Pegmatite in Lawler Peak granite .....	1420	1500
3. Pegmatite, Wickenburg, Ariz...	1160	1300
4. Pidlite Mine, Mora Co., N. M..	1330	1490
5. Granite, Sandia Mts., Albuquer- que, N. M.....	1350	1340
6. Harding Mine, Dixon, N. M...	1300	1300
7. Uncompahgre granite, Mesa Co., Colo. ....	1320	1320
8. Granite, Doyleville, Colo.....	1320	1310
9. Brown Derby pegmatite, Ohio City, Colo. ....	1330	1420
10. Granite, Sherman, Wyo.....	1420	1410
11. Silver Plume granite, Colo.....	....	1280

metamorphism in the Appalachian and Grenville Provinces.

Zircon ages, reported previously from the Lawler Peak and Quartz Creek granites, have been found to be discordant in such a way that the Laramide orogeny could have been partly responsible.

*Precambrian ages in Ontario.* The Laurentian Shield in central and eastern Canada and northern Michigan, Wisconsin, and Minnesota has long been recognized as a favorable area for the study of problems in Precambrian geochronology.

Approximately 2 million square miles of glaciated volcanic, plutonic, and sedimentary rocks are exposed. To the northwest, rocks of the Timiskiming-Keewatin Province are cut by pegmatites having ages of about 2600 million years. To the southeast in the Grenville Province, numerous intrusive igneous rocks have ages of about 1000 million years. Between these two provinces is another, narrower belt, the Huronian series, which is known to be younger than the Timiskiming-Keewatin rocks, but is of unknown relation to the Grenville rocks since the Huronian and Grenville are bounded by a fault zone.

There are, then, several reasons for making detailed age studies on rocks in the Laurentian Shield. Precambrian rocks are exposed over a great area, and they represent a long span of time, at least 1000 million to 2500 million years ago. There is a need to determine the relations of the Huronian to the Timiskiming-Keewatin series on an absolute time scale and, if possible, to fit the Grenville series into this sequence. Studies were commenced on these problems in the past year, with particular reference to the Huronian series. Valuable information has been obtained, although no conclusive solutions have resulted as yet.

Mica ages have been determined for a number of igneous and metamorphic rocks of known stratigraphic relation to the sedimentary rocks around Sudbury: the Huronian, Sudbury, and Keewatin series. Their absolute time sequence and their geographic extent have been among the major unsolved problems in Precambrian geology. The present studies have attempted to place limiting values on the ages of the various series by studying the ages of micas from rocks known to be older or younger than a particular series. This approach is necessary since there is no proven way at present to date the sediments directly.

The results obtained so far appear in tables 5, 6, and 7. Immediately evident are the numerous discordances between the

rubidium-strontium and potassium-argon ages for many of the micas, in sharp contrast to our past experience. Moreover, the few discordances found previously were of the type for which the potassium-argon age was less than the rubidium-strontium age; they were considered an indication of argon leakage. Several of the Sudbury micas give potassium-argon ages that are much greater than the rubidium-strontium

ages. As yet there is no explanation for these rather surprising inconsistencies.

Although the data contain a large number of discordant ages, some conclusions may be drawn. The Wavy Lake granite represents an outlier of igneous rock of the same age as the igneous rocks that intrude the Grenville sediments in the Grenville subprovince. The sediments called Huronian in this area appear to be more

TABLE 5. Age Determinations on Micas from the Sudbury District

Location and Sample	Stratigraphic Position	Age, million years	
		Rb-Sr	K-A
Wavy Lake granite.....	Intrudes Huronian .....	1075	1025
Sudbury gabbro .....	Intrudes lower Huronian.....	1325	1830
Sudbury breccia (matrix).....	Younger than Sudbury series and Copper Cliff rhyolite .....	1440	1870
Levack norite .....		1830	
Haleyburian lamprophyre .....	Pre-Cobalt (middle Huronian).....	2050	2160
Hearst pegmatite .....	Intrudes Keewatin .....	2595	2605
Round Lake lamprophyre.....	Pre-Huronian, Post-Keewatin .....	2600	2450
Round Lake batholith, granite.....	Pre-Huronian, Post-Keewatin.....	2640	2530
Timmins, granite .....	Pre-Huronian, Post-Keewatin .....	2470	2520

TABLE 6. Age Determinations on the Cutler Batholith  
(Intrudes the Sudbury series)

Sample		Age, million years	
		Rb-Sr	K-A
Pegmatite 1	Muscovite .....	1750	1440
	Feldspar .....	1760	1165
Pegmatite 2	Muscovite .....	1700	1420
Granite	Biotite .....	1325	1380

TABLE 7. Age Determinations on the Copper Cliff Rhyolite  
(Stratigraphic position uncertain)

Sample		Age, million years	
		Rb-Sr	K-A
Muscovite .....		1730	1390
Biotite .....		1220	2130
Feldspar .....		2360	1400

than 1300 million and less than 2600 million years old. They are actually less than 2150 million years old if the nearly concordant age obtained for the Haleyburian lamprophyre is significant and if the age of the mica from the lamprophyre does not represent a period of metamorphism subsequent to its formation. Since so many discordant ages have been found in this area, the 2150-million-year limit must be viewed with caution until further samples of the same age are found. The Sudbury series is older than 1400 million years because it is intruded by the Cutler batholith. It is possibly older than 1750 million years since most of the rubidium-strontium ages obtained from the Cutler give this value. The Keewatin series is older than 2600 million years. It should be emphasized that these conclusions apply only to these series as they are identified around Sudbury. The results cannot be extrapolated with certainty to form conclusions regard-



ing rocks called "Huronian" or "Keewatin" elsewhere.

The close proximity of the 2600-million-year-old rocks at Timmins and the Round Lake batholith at Kirkland Lake to the 1000-million-year-old rocks at Wavy Lake is interesting. Between these areas, only 150 miles apart, rocks of intermediate age occur, so that repeated igneous activity must have occurred in this rather restricted area. The large number of discordant ages found in the micas may bear some relation to this fact.

The 2600-million-year-old rocks listed in table 5 have about the same age as a number of intrusions found elsewhere. Rocks of this age have now been found in northern Wyoming, southern Montana, northern Minnesota, and southeastern Manitoba, as well as at Hearst and north of Sudbury in Ontario. Rocks with ages of 2600 million to 2700 million years on the continents of North America, Africa, and Australia are still the oldest reliably dated rocks. It seems probable that reliable dates for older rocks will be determined in the future, because these old rocks are intruded into sediments that must have been derived in turn from substantially older igneous rocks.

#### STUDY OF A POINT IN THE FOSSIL TIME SCALE

While serving as Fulbright lecturer to the University of Strasbourg during the academic year 1954-1955, Faul collected a suite of granitic rocks from the general area of the Hercynian Chain and some of its suspected outliers. Samples were taken in the Oslo area, the Harz Mountains, the Schwarzwald Mountains, the Vosges Mountains, the Alpine complex, and the Massif Central. Field work in each area was carried out in close co-operation with, and where possible in the company of, a local geologist who was particularly interested in the igneous petrology of the area. The field assistance of Drs. Barth, Denkel, Gjelsvik, Hügi, Roques, Siat, Wedepohl, Weil, Wenk, and Wimmenauer is gratefully acknowledged.

The large (30-lb or more) rock samples were crushed, ground, and separated into their principal mineral constituents with particular attention to zircon and mica. The separations were made at the institutes of mineralogy and physics at the University of Strasbourg.

Analyses of selected zircon samples for uranium, thorium, and lead and of some of the mica samples for potassium, rubidium, strontium, and argon have been made by the isotope dilution methods described in previous reports, as part of the co-operative program with the Department of Terrestrial Magnetism.

The zircon data obtained thus far are summarized in table 8. They show that all the zircons contain original lead, with the possible exception of the Oslo sample (which contains too little lead to determine whether or not original lead is present in significant proportion). In agreement with our previous experience, reliable age information cannot be obtained from zircons with appreciable common lead content, and these rocks cannot be dated by analyzing zircon alone.

As part of the zircon program, a suite of galenas from the Vosges Mountains was analyzed for lead isotopes (see table 9). As expected, the composition of these leads is very similar to that of the common leads from Germany, analyzed by Geiss, so that we cannot justifiably calculate the zircon data into concordance by accepting some unusual isotopic composition for the original lead they contain.

Rubidium-strontium and potassium-argon analyses of micas from some of these same rocks show a much more consistent picture (see table 10). Within the limits of error the age is the same for all the rocks, about 345 million years.

The stratigraphic age of these rocks is known to be pre-Westphalian (pre-middle Carboniferous), and they are usually assigned to the Dinantian (lower Carboniferous). According to the U. S. Geological Survey version of the Holmes time scale, the Carboniferous began 265 million years ago. By this time scale the present

measurements would place the Hercynian rocks in the middle Silurian. It follows that either the time scale is not correct here or the accepted stratigraphic assignment must be in error.

ured as well. In general, the new determinations have not altered the conclusions stated a year ago—that discordant isotopic ages are accompanied by the presence of common lead in the samples, and zircons

TABLE 8. Age Determinations on Zircons from the Hercynian Chain

Location	Pb (total), ppm	Pb (origi- nal), per cent	U, ppm	Th, ppm	Age, million years			
					U <sup>238</sup> / Pb <sup>206</sup>	U <sup>235</sup> / Pb <sup>207</sup>	Th <sup>232</sup> / Pb <sup>208</sup>	Pb <sup>207</sup> / Pb <sup>206</sup>
Natzwiller, Vosges .....	73	32	{ 989 991	534	292	...	263	.....
Col de la Grosse Pierre, Vosges..	166	20	2850	865	293	312	291	440 ± 60
Wembach, Schwarzwald .....	46	11	740	382	337	326	283	260 ± 60
Martinskapelle, Schwarzwald ..	163	59	1730	343	247	...	272	.....
Martinskapelle, Schwarzwald, leached with hot HCl.....	86	34	1251	345	292	...	...	.....
Halbmeil, Schwarzwald .....	78	40	784	177	374	...	285	.....
Oslo nordmarkite .....	{ 18 17	..	{ 363 365	{ 388 396	267	282	225	395 ± 60

Braces show duplicate determinations.  
Errors (standard deviations) are less than 2 per cent except where shown otherwise.  
When ages involving Pb<sup>207</sup> are not given, too much primary Pb<sup>207</sup> was present to permit accurate determination of radiogenic Pb<sup>207</sup>.

TABLE 9. Isotopic Composition of Galenas from the Vosges Area

Location	Isotopic Ratios				
	206/204	206/207	206/208	207/204	208/204
Ste. Marie .....	18.80	1.206	0.485	15.58	38.73
	± 0.14	± 0.005	± 0.008		
Musloch, Ste. Croix.....	18.37	1.186	0.482	15.49	38.08
	± 0.16	± 0.002	± 0.004		
La Croix .....	18.88	1.211	0.481	15.60	38.90
	± 0.27	± 0.007	± 0.001		
“Donner” mine .....	18.37	1.184	0.483	15.52	38.03
	± 0.05	± 0.007	± 0.003		
“Aurora” mine .....	18.65	1.189	0.481	15.68	38.73
	± 0.08	± 0.004	± 0.0015		
Steinbach .....	18.74	1.200	0.487	15.62	38.45
	± 0.065	± 0.0015	± 0.0015		
Wegscheid .....	18.24	1.157	0.467	15.76	39.00
	± 0.23	± 0.009	± 0.005		
Auxelles-Haut .....	18.72	1.190	0.483	15.72	38.77
	± 0.05	± 0.003	± 0.007		

The errors indicated are the observed mean deviations of usually about ten sets of ratios.

ZIRCON AGE WORK

Isotopic age determinations have been completed for six zircons, excluding those from the Hercynian Chain. In three, the ages of associated micas have been measured with no detectable common lead give concordant, or nearly concordant, isotopic ages. Among the zircons reported in table 11, those from Conway, Canada Hill, and Finland contained no detectable common



TABLE 10. Mica Age Results

Location	Age, million years	
	Rb-Sr	K-A
Vosges		
Lac Blanc .....	* { 340 346	336
Natzwiller .....	350	
Col de la Grosse Pierre.....	333	344
Schwarzwald		
Wembach .....	345	
Martinskapelle .....	341	
Halbmeil .....	334	
Sasbach-Walden .....	331	
Massif Central		
Royat .....	351	328

\* Duplicate determination.

at present for the cause of the relation between the common lead content of a zircon sample and the age results.

Our previous work showed that three zircons from the Grenville subprovince in Ontario gave concordant isotopic ages in spite of the fact that the samples varied greatly in crystal size and amount of radiation damage. The two zircons from the Fenno-Scandian Shield have likewise given quite satisfactory age results. The result for the Rapakivi granite is somewhat discordant, but not so discordant as the results found for zircons containing common lead, in which the  $U^{238}$ - $Pb^{206}$  and  $Pb^{207}$ - $Pb^{206}$  ages have differed by factors of nearly 2.

TABLE 11. New Age Results for Zircon and Associated Biotite

Location	Mineral	Age, million years					
		$U^{238}/Pb^{206}$	$U^{235}/Pb^{207}$	$Pb^{207}/Pb^{206}$	$Th^{232}/Pb^{208}$	$Rb^{87}/Sr^{87}$	$K^{40}/A^{40}$
Conway, N. H., granite	Zircon	187	184	140 ± 60	190		
	Biotite					185	182
Wichita Mts., Okla., pegmatite (zircon)*	Zircon <i>A</i>	520	527	550	506		
	Zircon <i>B</i>	514	522	550	493		
	Biotite					500	480
Bodom granite, Finland †	Zircon	1590	1625	1675	1540		
Rapakivi granite, Finland †	Zircon	1165	1350	1650	1050		
Canada Hill gneiss, Bear Mt., N. Y.	Zircon	1020	1060	1150			
	Biotite					1030	930
Hybla, Ontario, McDonald Mine	Cyrtolite	1350	1190	900	435		

\* Zircons *A* and *B* are separate zones separated from a single large crystal. *A* has uranium and thorium contents about six times those of *B* and correspondingly more radiation damage.  
† The Bodom and Rapakivi determinations were made by O. Kouvo at these laboratories.

lead and all give reasonably concordant ages. The Wichita Mountains samples are a notable exception to the generalization, for while they give nearly concordant ages the lead in sample *A* contains 10 per cent common lead and that in sample *B* contains 5 per cent common lead. These are the exceptions that have been found in the 16 zircons analyzed to date. The Hercynian zircons must be left out of the present discussions until studies can be completed in that area. No explanation can be given

The report of a year ago mentioned the observation that discordant isotopic ages were found for zircons from Precambrian granites from the Cordilleran System in western United States, where orogenies have occurred in more recent (Mesozoic) times. In contrast to the zircons, micas separated from the same granites appear to have preserved their ages through whatever events were responsible for the discordant zircon ages. One observation was made in the Appalachian Province to test further

the possible influence of orogenies on zircon age results. The Canada Hill gneiss from the Hudson highlands has been studied. The rock is of Precambrian age and has been folded during the Taconic orogeny. From past experience it was expected that the mica might preserve the original age of the rock but the zircon might give discordant age results which would have some connection with the orogeny. Actually, the biotite and zircon ages appear to have been relatively unaffected by the orogeny, although the potassium-argon age of the biotite is probably somewhat low.

*Geologic implications of the ages.* The Wichita Mountains biotite was separated from the Lugert granite, and the zircon came from a pegmatite in the same granite. The results from table 11 give an age of about 500 million years for the granite. This granite has been called "Precambrian" in published works by geologists. Recent discussions with Professor Clifford Merritt, of the University of Oklahoma, and Dr. William Ham, of the Oklahoma Geological Survey, indicate that some uncertainty attaches to this stratigraphic assignment. If the granite is in fact Precambrian, it would be the youngest Precambrian rock known and would thus be of considerable importance to the fossil time scale.

The northern and central Appalachian Mountains have provided many mica age measurements grouping at 300 to 350 million years, but the zircon and mica from

the Canada Hill gneiss in the heart of the chain are 1000 million years old, the same age found for a zircon in the Adirondack Mountains just to the west and for the igneous rocks that intrude the Grenville sediments in Ontario. It would appear that some parts of the Appalachian Mountain rock were not affected by the Taconic orogeny about 350 million years ago.

The Bodom and Rapakivi granites appear to be 1600 million to 1650 million years old. They are considered to be of post-Karelidic age.

#### ACKNOWLEDGMENTS

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Several of the general statements in this report are based on the data of our colleagues at the University of Minnesota, the Lamont Geological Observatory, the Massachusetts Institute of Technology, the University of Toronto, and the California Institute of Technology, as well as on our own data.

### SIMPLE ABSOLUTE MEASUREMENT TECHNIQUE FOR BETA RADIOACTIVITY; APPLICATION TO NATURALLY RADIOACTIVE RUBIDIUM

*W. F. Libby*

The earlier method of Suttle and Libby for routine simple absolute assay of solids did not specifically take account of the fact that the back-scattered radiation is somewhat softer than the original  $\beta$  radiation and depends in both intensity and softness on the atomic number of the back scatterer. This effect has been recognized during the course of the present research, and agree-

ment with the known standard  $\beta$  samples as furnished by the National Bureau of Standards and the Oak Ridge National Laboratory has been improved.

An important new finding is that the rough surface of a crystalline powder requires a larger correction for geometry than a smooth surface. This fact has been shown by direct calculation and proved



experimentally. The geometrical effect of the surface roughness of a powdered solid is most marked for soft  $\beta$  rays, for which the surface looks much rougher than for hard  $\beta$  rays. Empirically, for powders as ordinarily prepared, a half-thickness of about 7 mg/cm<sup>2</sup> seems to be a good dividing line;  $\beta$  radiations of smaller half-thickness require a geometry factor some 40 per cent larger than those of larger half-thickness. The geometry factor for hard  $\beta$ 's is the same one calculated for a smooth surface.

With these changes, the technique of measuring the absolute radioactivity of solids and liquids by placing them in a cylindrical position around an ordinary Geiger counter gives results agreeing with the true absolute assays within 5 per cent.

The technique was applied to the measurement of the half-life of naturally radioactive rubidium. The value found was  $50.7 \pm 2$  billion years, in good agreement both with that determined by Aldrich, Wetherill, Tilton, and Davis on old rocks by the uranium-lead method and with the latest value determined by Huster, Rausch, and Geese-Bahnisch by  $4\pi$  counting of very thin deposits of rubidium salts.

This technique should have wide application in the development of new uses of isotopes, particularly in introducing isotopes into the ordinary chemistry classroom.

#### THE METHOD

Suttle and Libby showed that, under conditions of cylindrical geometry in which the sample lies on the surface of a cylinder whose axis is identical with that of the Geiger counter used to measure the radiation,  $\beta$  radiations resulting from a single transition between two nuclear energy states are absorbed exponentially, even though the transition may be highly forbidden as in K<sup>40</sup>. The cylindrical position of the sample is essential for the control of the very large effects of  $\beta$ -ray scattering; it is for this reason that the popular end-window type of counter with its flat sample

does not give exponential absorption without special orifice windows to control the scattering. These ordinary counters can be used for absolute counting only with rigorous controls and exacting disposition of sample which present considerable difficulty in the usual laboratory; as a result, applications of isotopes that involve the use of absolute counting have not been generally made.

The fact that nearly exponential absorption curves can be obtained under certain conditions has long been known. As has been shown earlier, if the absorption of the radiation is exponential, the total self-absorption in an ordinary solid or liquid sample, which has finite thickness and therefore can be readily made and handled, can be easily calculated and the relation between the absolute disintegration rate and the observed count rate obtained. It was assumed that the effects of the self-scattered radiation would be encompassed in the geometrical constant  $G$  used in the formula. This point is examined in the present research.

Let  $\sigma$  = absolute specific radioactivity, disintegrations/min/mg of sample.

$1/\lambda_s$  = absorption coefficient of the radiation in the material of the sample, cm<sup>2</sup>/mg.

$1/\lambda_w$  = absorption coefficient in counter-wall material, cm<sup>2</sup>/mg.

$l$  = wall thickness of counter, including the air between the surface of the sample and the counter wall, mg/cm<sup>2</sup>.

$G$  = geometry factor, the ratio of  $4\pi$  to the average solid angle subtended by the inner surface of the cylindrical counter wall at the sample surface.

$x$  = sample thickness (less than saturation), mg/cm<sup>2</sup>.

$\bar{Z}$  = atomic number of the sample on a weight average basis.

$\eta$  = back-scattering coefficient for close geometry.

$A$  = area of sample, cm<sup>2</sup>.

Then, for a layer of sample at depth  $y$  ( $\text{mg}/\text{cm}^2$ ) below the top, and of thickness  $dy$ , the count rate will be:

$$dR = (A\sigma/G)(1 + \eta)e^{(-y/\lambda_s - l/\lambda_w)} dy \quad (1)$$

or, integrating over the sample thickness,

$$R = (A\sigma/G)\lambda_s(1 + \eta)(1 - e^{-x/\lambda_s})e^{-l/\lambda_w} \quad (1)'$$

Seliger has shown that the back-scattered radiation is of lower energy and softer in penetrating power than the original radiation, the softening depending on the angle of scattering as well as on the atomic number of the back-scattering material. Muller has very carefully studied the variation of back scattering with the atomic number of the material causing the back scattering under a particular set of geometrical conditions, with applications to analytical chemistry in mind. It is generally agreed that the back-scattered radiation is softer than the original, that for materials of  $\bar{Z}$  below 15 the factor by which the absorption coefficient of the back-scattered radiation measured under  $2\pi$  conditions is increased is about 2, and that for larger values of  $\bar{Z}$  the factor decreases essentially linearly to about 1.2 at atomic number 90. Therefore, for ordinary materials in which  $\bar{Z}$  is less than 15 we can write a new equation for the relation between the count rate and the absolute specific activity:

$$R \text{ (cpm)} = \frac{A\sigma\lambda_s}{G} \left(1 + \frac{\eta}{2} e^{-l/\lambda_w}\right) e^{-l/\lambda_w} \cdot (1 - e^{-x/\lambda_s}) \quad (1)''$$

On the right-hand side of this equation the first term in parentheses takes account of the fact that the counter wall and the air between the sample and the counter wall will absorb the back-scattered radiation more than they do the original. It also of course takes account of the magnitude of the back-scattered radiation.

The corresponding formula for larger values of  $\bar{Z}$  is easily obtained by replacing the coefficient 2 in this term by the appropriately smaller softening factor of the back-scattered radiation and including in the exponential term in the parentheses

the value of this new coefficient less 1. For samples that are thick with respect to  $\lambda_s$ , the last parenthetical factor (the saturation term) disappears, and for material of  $\bar{Z}$  less than 15, the softening of back scattering can be combined with the geometry factor into a new factor,  $G'$ , to obtain the formula of Suttle and Libby:

$$R = (A\sigma\lambda/G')e^{-l/\lambda_w} \quad (2)$$

Bothe and Danziger in theoretical studies of  $\beta$  absorption deduced the main features of the whole phenomenology, and their curve for  $\eta$  versus  $\bar{Z}$  agrees well with the experimental data of others. Since it is clear from the theory that the scattering effect for materials of low  $\bar{Z}$  should be particularly simple, it is not surprising that for these materials the very simple equation 2 is nearly as accurate as the more detailed equations 1' and 1''.

An empirical equation for  $\eta$  which has been used in this research and which fits quite well both the experimental and the theoretical relations between  $\eta$  and  $\bar{Z}$  for close geometry conditions approaching  $2\pi$  in the solid angle subtended by the counter is

$$\eta = 0.65(1 - e^{-\bar{Z}/35}) \quad (3)$$

The absorption coefficient,  $1/\lambda$ , has been found to depend on the maximum energy of the  $\beta$  spectrum,  $E$ , in the following way:

$$\lambda \text{ (mg}/\text{cm}^2) = 55E^{3/2} \quad (4)$$

or

$$l_{1/2} \text{ (mg}/\text{cm}^2) = 38E^{3/2} \quad (4)'$$

where  $l_{1/2}$  is the half-thickness in aluminum, and  $\lambda$  similarly refers to the reciprocal of the absorption coefficient in aluminum.

Lerch has shown that  $\lambda$ , the reciprocal of the absorption coefficient, depends on the average atomic weight,  $M$ , of the absorbing medium. The relation is:

$$\lambda_M = \lambda_0/[1 + (M/100)] \quad (5)$$

Equation 5 has been used in this research, and figure 15 shows the adequate degree to which it fits the experimental data for  $\lambda$  given in table 12.



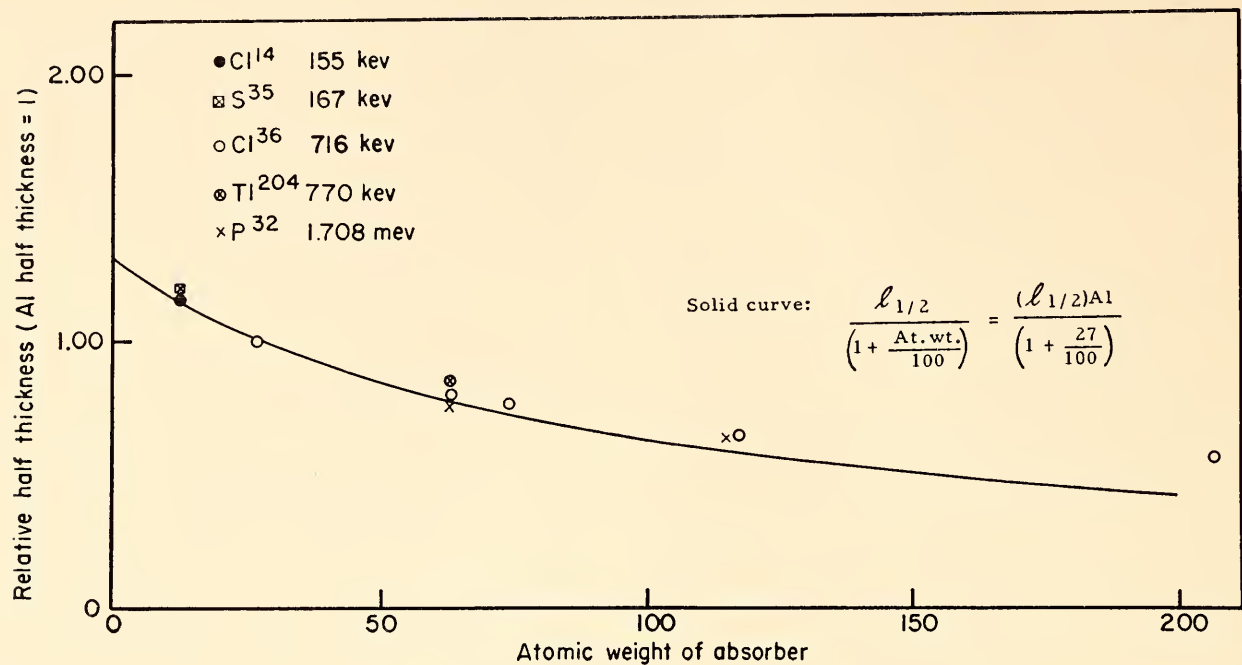


Fig. 15. Half-thickness versus atomic weight of absorber.

TABLE 12. Absorption Data

Isotope	Maximum Energy of $\beta$ Spectrum, Mev	Absorbing Material	Half-Thickness, mg/cm <sup>2</sup>	Reciprocal of Absorption Coefficient, mg/cm <sup>2</sup>
T	0.0189	He	0.050	0.0720
Zr <sup>93</sup>	0.060	Al	0.35	0.506
Sm <sup>151</sup>	0.0755	Al	0.63	0.91
C <sup>14</sup>	0.155	Al	1.9	2.74
S <sup>35*</sup>	0.167	Mylar plastic*	2.2*	3.16*
		Al*	2.3*	3.3*
		Mylar*	2.7*	3.9*
Rb <sup>87</sup>	0.270	Al	4.85	7.0
Ca <sup>45*</sup>	0.255	Al*	4.9*	7.1*
Tc <sup>99</sup>	0.296	Al	6.09	8.8
Tl <sup>204</sup>	0.762	Al	22	32
Cl <sup>36*</sup>	0.716	Al*	32*	46*
		Cu*	26*	37*
		Sn*	21*	30*
		Pb*	18*	26*
K <sup>40</sup>	1.36	Al	67	96
P <sup>32</sup>	1.708	Al	84	122
		Cu*	60*	86*
		Sn*	50*	72*
Y <sup>90*</sup>	2.275	Al*	130*	189*

\* New data.

Of course, the fact that the back-scattered radiation is softened relative to the original radiation means strictly that the absorption curve cannot be entirely exponential for the thinnest layers of absorber, since the absorption relation must be as given in equation 1". The verification of this statement is to be seen in figure 16, which shows actual absorption curves of various soft  $\beta$  emitters taken in the screen wall counter, which allows the very softest radiation to be measured. Here we see that

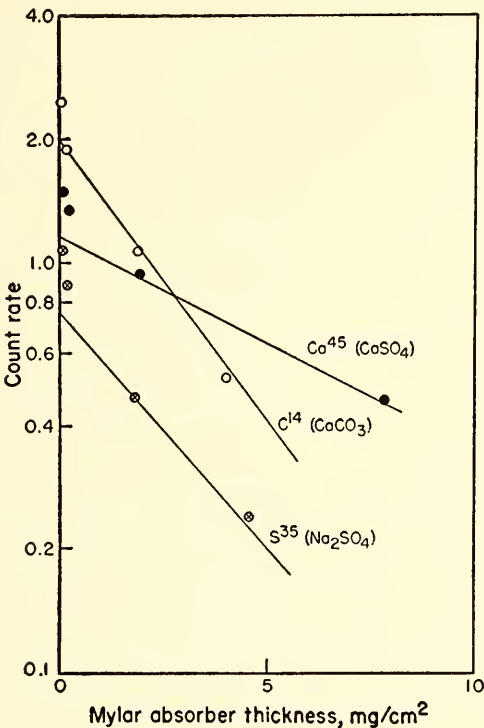


Fig. 16. Absorption curves for bare soft  $\beta$ 's. Sources in screen-wall counter.

there is a soft component that is quickly absorbed out, and then the long normal exponential absorption curve is left, which is the only curve observed with Geiger counters of usual wall thickness. Therefore, the absorption is not strictly exponential for soft  $\beta$  emitters. Exponential curves will be found for hard  $\beta$  emitters, for in this case the back-scattered radiation is lost in the large percentage of hard radiation that is present from thick solids. Clearly, however, for hard radiations and thin sources the absorption term should not be strictly exponential.

The absorption curves for the naturally

radioactive element rubidium observed in the earlier work of Suttle and Libby showed a nonexponential character in a way that was difficult to understand at that time. This, we now see, is the result of the large  $\bar{Z}$  for rubidium compounds in increasing the back-scattering coefficient  $\eta$ , and of the fact that the radiation from rubidium is soft (cf. table 12). For counters with walls as thin as 2 mg/cm<sup>2</sup>, the absorption curves normally are exponential as

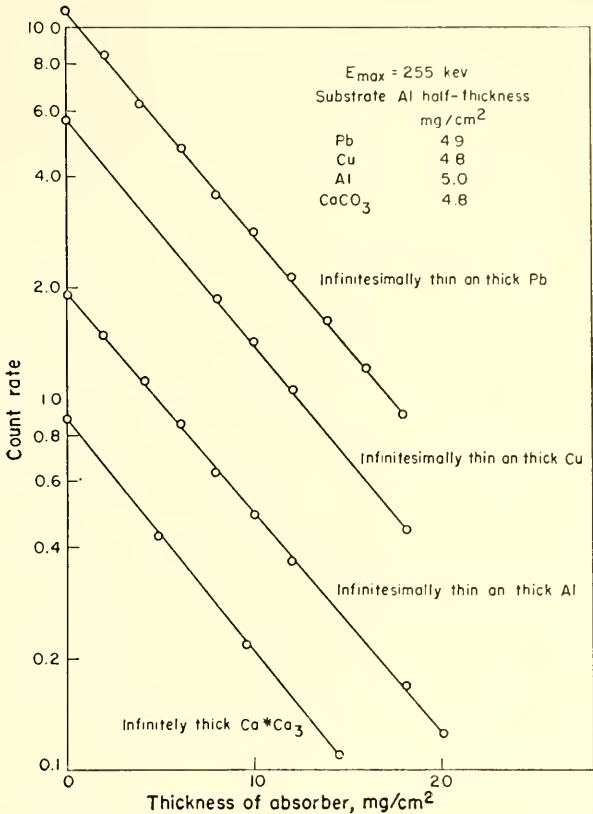


Fig. 17.  $\text{Ca}^{45}$  absorption curves in aluminum.

shown in figure 17, in agreement with figure 18 for the corresponding values of absorber plus counter wall thickness.

The value of  $G$ , the geometry factor in the full equation 1", is precisely the ratio of  $4\pi$  to the average solid angle subtended at the sample surface by the inner wall of the counter. If the counter is long relative to the sample, and if the radius of the inner wall is  $\rho$  and the radius of the sample surface relative to the counter wire is  $r$ , then it can be shown that

$$G = \pi / [\pi/2 - \cos^{-1} (\rho/r)] \tag{6}$$

is the equation for the geometrical value of  $G$ .



A very important question had to be answered in the course of the attempt to apply this simple absolute assay technique to soft  $\beta$  emitters. It was found that the results obtained for the specific radioactivity were normally and uniformly about 30 per cent low for all isotopes with  $\lambda$  values below about 10. The effect was the purely geometrical one of the roughness of the surface of a crystalline powder. For soft  $\beta$  rays for which the range in the solid was less than the thickness of the crystals, the only radiation escaping was from the surfaces of the crystals. With harder  $\beta$ 's, however, the entire crystal emitted and the surface-effect roughness disappeared.

The effect of roughness of the sample is important. The expression (6) is applicable only to a smooth sample constituting the wall of a cylinder of inner radius  $r$ , or a portion of the wall of such a cylinder. Consider a normal crystalline powder consisting of cubes  $50\ \mu$  on edge. If the density were  $2\ \text{gm/cm}^3$ , then the cube edge would correspond to an  $x$  value in equations 1' and 1'' of  $10\ \text{mg/cm}^2$ . Therefore, we see immediately that, for hard  $\beta$  emitters with  $\lambda$  values larger than  $10\ \text{mg/cm}^2$ , the surface of the solid powder of randomly oriented cubes would appear to be smooth and the powder would have a  $G$  value close to that for a smooth surface as given by equation 6. For soft  $\beta$  emitters, on the other hand, only the surfaces of the crystals can emit, and the surface therefore must appear rough. The fact that roughness causes a reduction in the total outward flux of radiation relative to that from a true smooth cylindrical surface of the same material at the same specific radioactivity may not be obvious, but detailed calculation for various likely powders, such as randomly oriented cubes or hexagonally packed spheres, shows that this is a general result and that the magnitude of the effect agrees with the results on the various  $\beta$ -radiation standards obtained from the National Bureau of Standards (through the kindness of Dr. W. B. Mann) and the Oak Ridge National Laboratory (through the

kindness of Mr. S. A. Reynolds). By means of equations 1', 1'', and 2,  $G$  was calculated from the observed count rate  $R$  and the known specific radioactivity  $\sigma$ . The results are given in table 13.

The counter used consisted of a thin metallized plastic cylindrical wall inflated by the counting gas gently flowing through at pressure slightly in excess of atmospheric. The counter had a wall thickness of  $1.82\ \text{mg/cm}^2$ , and the sample was placed around the counter on the inner surface of a plastic cylinder on which was placed a sheet of rubber  $1.5\ \text{mm}$  thick with a square or circular hole of accurately known area punched in it. The distance between the counter wall and the surface of the sample was  $0.27\ \text{mg/cm}^2$  of air. By the use of the rubber sheet, the sample area was accurately known. The sample powder was placed in the recess of the rubber sheet and smoothed with a spatula. Under these conditions the counter wall radius,  $\rho$ , was  $1.5\ \text{cm}$  and the inner sample surface radius was  $1.8\ \text{cm}$ .

The larger  $G$  factor for soft radiation as shown in table 13 was further established experimentally by making a finely divided  $\text{Na}_2\text{CO}_3$  which had  $\text{C}^{14}$  in it. This was done by powdering  $\text{Na}_2\text{C}^*\text{O}_3 \cdot \text{H}_2\text{O}$  and then dehydrating it at low oven temperatures so that sintering did not occur. Under these conditions the value of  $G$  was the smaller one for hard  $\beta$ 's rather than the larger one found for the same salt before dehydration, as shown in table 13. In all other cases for soft  $\beta$  radiations the  $G$  was higher, and the average for all soft  $\beta$ 's with  $\lambda$  values less than 10 was 3.9 versus 2.72 for the hard  $\beta$ 's. Table 13 gives the final  $G$  values as determined according to the three equations 1', 1'', or 2.

It is clear, of course, that the necessity for deciding which value of  $G$  applies to a particular solid sample (and  $\beta$  radioactivity) being measured is a point of concern. How can one tell? For  $\beta$ 's with  $\lambda$  values well above  $10\ \text{mg/cm}^2$ , the roughness necessary for the larger value of  $G$  will be easily visible and easily destroyed by

grinding with a mortar and pestle. Therefore, for this class of radioactivity successive measurements after grinding will bring the count rate to a constant high value independent of the degree of fineness of the solid which is characteristic of the smooth surface and the lower *G* value.

For the softer β's, however, it is necessary to do the opposite—to grow the crys-

The procedure for converting the β standards, which were solutions of very high specific activity, to solid form for measurement was to add a solution of an appropriate salt to a known volume of the standard, mix, evaporate or precipitate chemically, and grind the resultant solid. Sometimes it was difficult to obtain solids that were chemically identical with the

TABLE 13. Experimental Geometry Factors, *G*

Isotope	Substrate	λ, mg/cm <sup>2</sup>	<i>G</i>		
			Fully Corrected for Back Scattering and Softening, equation 1''	Partly Corrected, equation 1'	No Back- Scattering Correction, equation 2
P <sup>32</sup> .....	Na <sub>2</sub> SO <sub>4</sub>	129	2.65 ± 0.02	2.73 ± 0.02	2.33 ± 0.02
	(NH <sub>4</sub> )H <sub>2</sub> PO <sub>4</sub>	141	2.87 ± 0.03	2.85 ± 0.03	2.02 ± 0.03
K <sup>40</sup> .....	K <sub>2</sub> SO <sub>4</sub>	98	2.73 ± 0.06	2.83 ± 0.06	2.28 ± 0.05
Cl <sup>36</sup> .....	NaCl	45	2.85 ± 0.03	2.97 ± 0.03	2.43 ± 0.03
	AgCl	34	2.74 ± 0.03	2.86 ± 0.03	1.99 ± 0.03
	BaCl <sub>2</sub>	34.4	2.61 ± 0.03	2.71 ± 0.03	1.87 ± 0.03
Tl <sup>204</sup> .....	TlCO <sub>2</sub> H	27.1	3.09 ± 0.04	3.11 ± 0.05	2.01 ± 0.05
Average for λ > 10 .....			2.72	2.82	2.20
Ca <sup>45</sup> .....	CaCO <sub>3</sub>	7.5	4.12 ± 0.04	4.41 ± 0.05	3.72 ± 0.04
	CaSO <sub>4</sub> · 2H <sub>2</sub> O	7.9	4.18 ± 0.04	4.47 ± 0.04	3.76 ± 0.04
	CaO	7.0	4.28 ± 0.04	4.60 ± 0.04	3.71 ± 0.04
	CaSO <sub>4</sub>	7.4	3.73 ± 0.03	4.01 ± 0.03	3.28 ± 0.03
S <sup>35</sup> .....	Na <sub>2</sub> SO <sub>4</sub>	3.5	3.46 ± 0.3	3.78 ± 0.3	3.16 ± 0.3
	BaSO <sub>4</sub>	3.0	4.4 ± 0.2	5.0 ± 0.3	3.5 ± 0.3
C <sup>14</sup> .....	CaCO <sub>3</sub>	2.9	3.4 ± 0.1	3.76 ± 0.1	3.16 ± 0.1
	Na <sub>2</sub> CO <sub>3</sub> · H <sub>2</sub> O	3.1	4.15 ± 0.16	4.56 ± 0.18	3.95 ± 0.1
	Very fine Na <sub>2</sub> CO <sub>3</sub>	3.0	2.55 ± 0.08	2.78 ± 0.09	2.44 ± 0.09
Average for λ < 10 (omitting fine Na <sub>2</sub> CO <sub>3</sub> ) .....			3.9	4.2	3.5

tals larger and larger by sintering or other device, and thus to reach a constant count rate independent of crystalline size. Any doubt can be settled by a cursory examination with a microscope, the relative magnitude of the crystal size and λ being borne in mind. It appears that the soft β geometry factors for various powders are essentially the same, as can be seen in table 13, though there is some evidence of scatter, which could be due to the size or shape of the particular crystals.

radioactive molecules, and attempts were made to use substitutes with which the radioactive species was likely to form mixed crystals. For the harder β radiations, the requirement that mixed crystals be formed seemed to be less necessary. For example, radioactive phosphate containing P<sup>32</sup> (λ<sub>A1</sub> = 122 mg/cm<sup>2</sup>) was measured on Na<sub>2</sub>SO<sub>4</sub> powder. It seems unlikely that any substrate not chemically identical can be used in the case of soft β's.



RESULTS

In table 14 are shown the results of the application of the method to a series of  $\beta$ -radiation standards furnished by the National Bureau of Standards and the Oak Ridge National Laboratory. From these data it appears that the method is good to about 5 per cent. Equation 2, which is the simplest, does nearly as well, except for the large atomic numbers, as the more complete equations 1' and 1".

was applied. The results are given in table 15.

In calculating the final error for the half-life as determined on the three rubidium samples, the average deviation of 3.3 per cent for equation 1" as applied to soft  $\beta$ 's with a  $G$  value of 3.9 was used. The average deviation of 9.5 per cent for a single determination as given in table 14 was divided by the square root of the number of determinations to determine

TABLE 14. Results for Standard Sources  
(Per cent deviation) calculation

Isotope and Substrate		Fully Corrected, equation 1''		Partially Corrected, equation 1'		Uncorrected for Back Scattering, equation 2	
P <sup>32</sup> (NBS)	Na <sub>2</sub> SO <sub>4</sub> .....	+1.1		+0.7		+5.5	
	(NH <sub>4</sub> )H <sub>2</sub> PO <sub>4</sub> .....	+5.6		+1.2		-8.0	
Cl <sup>36</sup> (ORNL)	NaCl .....	+4.7	ave. 3.9	+5.4	ave. 4.4	+10.5	ave. 8.3
	AgCl .....	+0.7		+1.5		-9.4	
	BaCl <sub>2</sub> .....	-4.0		-3.9		-15	
Tl <sup>204</sup> (NBS)	TlCO <sub>2</sub> H .....	+13.6		+10.4		-8.7	
Ca <sup>45</sup> (ORNL)	CaCO <sub>3</sub> .....	+5.6		+5.1		+6.5	
	CaSO <sub>4</sub> ·2H <sub>2</sub> O .....	+7.1		+6.5		+7.6	
	CaO .....	+9.6		+9.5		-6.1	
	CaSO <sub>4</sub> .....	-4.4		-4.5		-6.3	
S <sup>35</sup> (NBS)	Na <sub>2</sub> SO <sub>4</sub> .....	-11	ave. 9.5	-10	ave. 9.8	-8.6	ave. 7.8
	BaSO <sub>4</sub> .....	+13		+20		+0.3	
C <sup>14</sup> (NBS)	CaCO <sub>3</sub> .....	-13		-10		-9.7	
	Na <sub>2</sub> CO <sub>3</sub> ·H <sub>2</sub> O .....	+6.4		+7.4		+13	

APPLICATION TO NATURALLY RADIOACTIVE RUBIDIUM

Aldrich, Wetherill, Tilton, and Davis compared the ratio of radiogenic Sr<sup>87</sup> to Rb<sup>87</sup> found in several minerals differing in rubidium content in rocks of known age as determined by the uranium-lead method. They calculated the half-life of natural radioactive Rb<sup>87</sup> to be 50±2 billion years. Strassman and Walling found 63 billion by a similar method. Because of the importance of this determination to geochronology, the technique described above

the average deviation of the mean from the true value. The agreement among the three different rubidium samples in table 15 indicates that the error of the determination is the error in the determination of  $G$ , which as explained should be 3.3 per cent.

GENERAL APPLICATIONS

Equation 2 certainly is simple enough for use in high-school laboratory experiments. In this way isotopes of real chemical interest, convenient lifetime, and low

enough specific activity to be completely safe can be introduced into the ordinary high-school chemistry course. Among these isotopes are C<sup>14</sup>, Cl<sup>36</sup>, S<sup>35</sup>, and Ca<sup>45</sup>. Thus the radioactive forms of acetic acid, hydrochloric acid, sulfuric acid, and the calcium salts can be placed on the reagent shelves and the label can carry the specific activity, so that the students by using a known volume can introduce a known amount of radioactivity, and by subsequent absolute counting of the various solids produced in the experiment can calculate an isotopic balance to compare with the ordinary material balance. This technique makes possible the ready application of isotopic dilution techniques.

In industrial applications the possibility of keeping an analytical check on a known amount of C<sup>14</sup> added in appropriate chemical form at the beginning of, or during, an industrial organic chemical process certainly affords many opportunities for controlling the process. The labeling of a particular constituent of the crude feed for an oil refinery for a fixed period would make possible a detailed examination of the flow rates and patterns throughout the plant, e.g. the contribution of this constituent to coke in the catalytic crackers, the completeness of the burn-off of the coke from this constituent in the burn-off cycle, or the general holdup in various stages in the plant.

TABLE 15. Determination of the Half-Life of Rb<sup>87</sup>

Sample	$\lambda$ , mg/cm <sup>2</sup>	$\eta$	Specific Activity, dpm/mg	Half-Life, billion years
RbCl ..... Purified by Dr. Suttle	3.85	0.384	53.1 ± 1	49.6 ± 1
RbCl ..... Johnson, Mattie & Co., London; spectrographically pure	3.85	0.384	51.7 ± 0.8	51.0 ± 0.8
Rb <sub>2</sub> CO <sub>3</sub> .....	3.83	0.361	54.0 ± 0.5	51.1 ± 0.5
Average .....				50.7 ± 2

PALEOBIOCHEMISTRY

EFFECTS OF ULTRAVIOLET LIGHT ON THE  
"PRIMITIVE ENVIRONMENT"

P. H. Abelson

The origin of life is a topic of transcending interest which has drawn the attention of many investigators. Many of them have sought to isolate a part of the problem both crucial and capable of solution—the synthesis of biological building blocks, especially amino acids, from simple chemicals of the environment. To accomplish these reactions a number of energy sources have been employed, including  $\alpha$ ,  $\beta$ , and  $\gamma$  radiation and electrical discharges. A variety of test chemicals has been employed, many of which make convenient experimental objects but could hardly have existed in substantial quantities on the primitive earth.

It is difficult to be certain concerning processes that might have occurred three billion years ago. It is feasible, however, to set some limits on the areas of permissible speculations. An analysis of the nature of the primitive environment could be expected to point up the importance of a few key compounds and reactions. Study of the effects of energy on these substances might then disclose reactions of major importance on the primitive earth.

The relative scarcity of the gases neon, argon, krypton, and xenon in our present atmosphere is a most significant phenomenon. The abundance curves of isotopes of the various elements are fairly well known, and neon has been observed as an important constituent of stellar atmospheres while being scarce on earth. Brown



and Suess have estimated that neon is present on our planet to an extent only  $10^{-10}$  of probable cosmic abundance, and that similarly argon, krypton, and xenon are relatively absent. We do not know the nature of the processes by which the earth was formed, whether these inert gases were lost during the accumulation period or subsequently; but at any rate they were lost, and it seems reasonable that such other volatile constituents as hydrogen, nitrogen, methane, and carbon monoxide would also have been lost at the same time.

Rubey has calculated the amounts of volatiles that have appeared in the atmosphere, hydrosphere, or biosphere since weathering first began. He advances many

TABLE 16. Inventory of Organic Matter

Element	Grams
Carbon	$68 \times 10^{20}$
Oxygen	$25 \times 10^{20}$
Hydrogen	$9.6 \times 10^{20}$
Oxygen needed to burn to $\text{CO}_2 + \text{H}_2\text{O}$	$235 \times 10^{20}$

powerful arguments that these volatiles were not originally present on the surface of the earth but came from the interior of the earth through a gradual degassing process. Of interest to the present argument are data that he has provided on the inventory of organic matter in sediments, shown in table 16. These permit construction of an oxidation-reduction balance. To measure the reducing side of the ledger, the amount of oxygen required to burn this organic matter to water and carbon dioxide can be calculated. The amount of oxygen in the atmosphere at present and that consumed in the oxidations of ferrous to ferric iron and sulfur to oxidized sulfur may be noted in table 17. Considerable uncertainty attaches to the amount of oxygen consumed in oxidation of sulfur, since the relative proportions of the original forms of this substance are not known; the value quoted represents an upper limit. There is not sufficient

oxygen or oxidation to match the reduced substances in the sediments. It is possible that this unaccounted-for oxygen was consumed in the oxidation of carbon monoxide and hydrogen issuing from volcanoes. The reducing nature of these gases is a consequence of the physical-chemical equilibria of water and carbon dioxide with the reduced iron compounds and possibly other substances.

A calculation shows that 3 atm of hydrogen would be in equilibrium with 1000 atm of water at  $1200^\circ \text{K}$ , and that at the same temperature 1 atm of hydrogen would be in equilibrium with 100 atm of water

TABLE 17. Inventory of Oxygen

$\text{O}_2$	Grams
In atmosphere	$12 \times 10^{20}$
Consumed in oxidation $\text{FeO} \rightarrow \text{Fe}_2\text{O}_3$	$14 \times 10^{20}$
Consumed in oxidation $\text{S} \rightarrow \text{SO}_3$	$41 \times 10^{20}$
Total accounted for	$67 \times 10^{20}$
Unaccounted for	$168 \times 10^{20}$
Total	$235 \times 10^{20}$

along the wüstite, fayalite, and magnetite join. The equations governing equilibria involving carbon dioxide, carbon monoxide, water, and hydrogen in the presence of fayalite, wüstite, and magnetite may be combined to eliminate the common oxygen component. As a result there can be obtained an equation involving the equilibrium of water, carbon dioxide, hydrogen, and carbon monoxide. If the volatile constituents that have appeared at the surface were in equilibrium with one another, and with a basaltic crust, the original chemical form of some of the constituents can be estimated, taking into account the oxidation-reduction balance. Results can be noted in table 18, where it is apparent that, since water is the major volatile that has been released, hydrogen was the major reduced substance accompanying such a mixture. Later it will

be seen that a highly important feature of the composition of these volatiles is the comparative amounts of carbon dioxide and hydrogen.

Relatively soon after the earth was formed it cooled to about its present temperature. Hence, only a very small proportion of water remained in the atmosphere. The oldest rocks that have been dated contain the same minerals as those found today. Weathering of these rocks led to sediments similar in composition to those formed recently. There are arguments and evidence making it reasonable to assume that the pH of the primitive oceans was not much different from that today. As carbon dioxide was liberated most of it dissolved in those waters, leav-

TABLE 18. Composition of Volatiles on the Basis of Equilibrium Conditions

Gas	Moles
H <sub>2</sub> O	920 × 10 <sup>20</sup>
H <sub>2</sub>	10.3 × 10 <sup>20</sup>
CO <sub>2</sub>	21 × 10 <sup>20</sup>
CO	0.24 × 10 <sup>20</sup>
N <sub>2</sub>	1.5 × 10 <sup>20</sup>

ing hydrogen, nitrogen, and carbon monoxide as the principal constituents of the atmosphere. This mixture was altered further through the action of various forms of energy.

In attempts to set up models for production of compounds in the primitive environment, several different kinds of energy sources have been tried. Earlier at the University of California the cyclotron α-particle beam was employed. Recently, β and γ radiation have been used as sources. The experiments of Stanley Miller have demonstrated the production of amino acids in an environment of methane, ammonia, and water as a result of the action of an electric discharge. Abelson has confirmed these findings and extended them by testing the effects of electrical discharges on twenty other mixtures of gases, including CO, N<sub>2</sub>, H<sub>2</sub>, H<sub>2</sub>O; CO<sub>2</sub>, NH<sub>3</sub>, H<sub>2</sub>,

H<sub>2</sub>O. A wide variety of compositions yielded mixtures of amino acids.

Analysis, however, shows that corpuscular radiation and electrical discharge are relatively limited as sources in comparison with the energy coming in sunlight. In table 19 is shown the relative energy available per year. It can be noted that energy from sunlight far exceeds that from other kinds of sources, lightning is relatively insignificant, and cosmic rays are of very little consequence in terms of their energy content. Levels of radiation due to natural

TABLE 19. Energy Input to Earth

Source of Energy	cal/cm <sup>2</sup> /yr
Sunlight	260,000
Lightning	0.9
Cosmic rays	1.5 × 10 <sup>-3</sup>

TABLE 20. Rocket Measurements by the Naval Research Laboratory of Energy Incident on Earth

λ, A	Energy, watts/cm <sup>2</sup> /μ
4500	0.22
3000	0.061
2800	0.024
2600	0.013
2400	0.0058
2200	0.003

radioactivity are variable, but in many areas are about equivalent to those due to cosmic rays.

Only part of the sun's energy is in the ultraviolet region, but this portion is particularly effective in causing chemical transformations. Rocket experiments carried on by the Naval Research Laboratory have measured the energy incident upon the top of the atmosphere with results shown in table 20. From these data it can be estimated that 468 cal/cm<sup>2</sup> of wavelengths shorter than 2540 A fall on the top of the atmosphere annually. Such radiation is absorbed by carbon dioxide. Simultaneously 19 cal/cm<sup>2</sup> of wavelengths



shorter than 1800 Å strike the atmosphere. This radiation can be absorbed by water. Carbon monoxide exhibits some slight band absorption at 2056 Å, but major interaction begins at 1546 Å. Only 5.5 cal/cm<sup>2</sup>/yr are found in shorter radiation.

Another way of assessing the possible role of ultraviolet radiation is to match annual production of chemicals and radiation. Such a comparison may be noted in table 21, where it is evident that the energy available far exceeds the annual production of chemicals.

Ultraviolet light decomposes carbon dioxide to carbon monoxide plus atomic oxygen. In the presence of hydrogen, atomic oxygen reacts to form OH+H. OH in turn reacts with hydrogen to form

TABLE 21. Annual Production of Chemicals and Energy per Square Centimeter

Å	Quanta	Moles
$\lambda < 2900$	$1.4 \times 10^{22}$	CO <sub>2</sub> $8 \times 10^{16}$
$\lambda < 2540$	$2.3 \times 10^{21}$	CO $9 \times 10^{14}$
$\lambda = 1100-1345$	$3 \times 10^{18}$	N <sub>2</sub> $6 \times 10^{15}$
		H <sub>2</sub> $4 \times 10^{16}$

water plus more atomic hydrogen. One may speculate that, as carbon dioxide of the primitive atmosphere was used up, being split into carbon monoxide plus oxygen, the partial pressure of carbon dioxide was maintained by the carbonate buffering system of the oceans. As long as there were large amounts of molecular hydrogen in the atmosphere, the oxygen liberated by the breakup of carbon dioxide would be converted into water. However, after most of the hydrogen had been used up, small amounts of free oxygen would appear, and they would recombine with carbon monoxide or any other reducing substance. Carbon dioxide must have acted as a sort of buffer in the oxidation-reduction system, guaranteeing that the atmosphere could never have been very reducing. It seems impossible to visualize any great concentration of substances such as methane present together with carbon dioxide.

The values given by Rubey indicate that in the last 3 billion years considerably more carbon dioxide has been liberated than is needed to supply oxygen to react with all the hydrogen or other reducing substances.

In an atmosphere consisting predominantly of carbon monoxide, nitrogen, and hydrogen, the action of short ultraviolet light could lead to a number of additional substances, including ammonia and hydrogen cyanide. Under any reasonable assumptions of the nature of the primitive oceans, all ammonia and hydrogen cyanide would be found in the aqueous phase with almost none remaining in the atmosphere. Carbon monoxide is slightly soluble in water, being slowly converted into formate. The interactions of ultraviolet light with the atmosphere and subsequent absorption of products in the oceans thus would modify the composition of both atmosphere and oceans. Later in this report some effects of ultraviolet light on the substances dissolved in the oceans will be considered.

In enumerating the kinds of organic compounds, formation of which would be crucial to the creation of building blocks essential for life, one is impressed by the possible important role of the aldehydes. This class of compounds could serve as important materials in the building-up of long carbon chains through reactions that can occur in the aqueous phase. No other set of organic substances can perform in quite such a manner. Strecker in 1850 found that, if formaldehyde, hydrogen cyanide, and ammonia are all simultaneously present in solution, glycinonitrile is formed. This substance subsequently is hydrolyzed by water to form the amino acid glycine. The Strecker synthesis is not limited to formaldehyde but may be carried out with other aldehydes. It is interesting that this type of synthesis gives rise to  $\alpha$ -amino acids, which are the principal type of amino acids used in biological processes.

It seemed important to determine whether ultraviolet light acting on for-

mate could lead to the extremely important substance formaldehyde. In alkaline solutions formate has little absorption at wavelengths longer than 2540 Å; above 2700 Å it is virtually transparent. It was recalled that iron is a ubiquitous constituent of the crust, and that, if there was little or no oxygen in the atmosphere, appreciable concentrations of ferrous iron might be present in solution. A 1 M solution of formate containing 0.004 M ferrous iron at a pH of 8.3 was irradiated, in an evacuated vessel, by ultraviolet light of wavelength 2536 Å. After 1 hour, a non-condensable gas was noted, and formaldehyde could be detected in the solution. At the end of 3 hours, approximately three times as much noncondensable gas was present but the same amount of formaldehyde was determined. In order to obtain a better measure of the rate of formation of formaldehyde, experiments were performed using a trapping mechanism designed to take formaldehyde out of the scene of destruction. For this purpose the Strecker synthesis was employed. Solutions 0.4 M in formate, 0.4 M in ammonium hydroxide, 0.2 M in sodium cyanide, and 0.0016 M in ferrous sulfate were irradiated for periods of an hour to several days, and the resulting glycinonitrile was hydrolyzed and determined as glycine. Relatively large quantities of this amino acid were found.

In another series of experiments the hydantoin reaction was employed as the trapping mechanism. For this purpose a solution 0.1 M in ammonium carbonate, 0.1 M in formate, 0.1 M in sodium cyanide, and 0.001 M in ferrous sulfate was irradiated for 2 days with 2536 Å radiation. The resulting hydantoin was hydrolyzed with NaOH, and glycine was isolated. A 10 per cent yield based on formate was obtained.

An important series of reactions is the condensation of aldehyde and cyanide followed by hydrolysis. The kinetics of the first of these two reactions has been studied using initial concentrations of 10<sup>-3</sup> M cy-

nide and 10<sup>-4</sup> M formaldehyde. Some results are shown in table 22. The reaction proceeds rapidly until the concentration of formaldehyde drops to 10<sup>-7</sup> M or 3 parts in 10<sup>9</sup>. The effect of pH on the reaction was also investigated. The optimum pH for the reaction was found to lie in the range 8 to 9.5. The reaction thus proceeds best at a pH near that of sea water. Hydrolysis of glycolonitrile to glycolic acid is an irreversible reaction which progresses at a moderate rate at 20° C. With the appearance of glycolic acid the stage is set for further synthesis. Irradiation by ultraviolet light again could yield an aldehyde;

TABLE 22. Reaction Kinetics at 20° C  
$$\text{HCN} + \text{CH}_2\text{O} \xrightleftharpoons{\text{H}_2} \text{HOC}-\text{C}\equiv\text{N}$$
  
10<sup>-3</sup> M NaCN, 1.05 × 10<sup>-4</sup> M CH<sub>2</sub>O, pH 8.1

Time, min	CH <sub>2</sub> O, 10 <sup>-4</sup> mole	Time, min	CH <sub>2</sub> O, 10 <sup>-4</sup> mole
0	1.05	6.0	0.100
1.1	0.64	7.0	0.078
2.1	0.433	9.0	0.035
3.1	0.320	10.0	0.025
4.0	0.207	12.0	0.010
5.0	0.147	20.0	0.001

evidence for this step is discussed later. Condensation with cyanide would result in a dihydroxynitrile, glyconitrile. A series of condensations followed by hydrolysis could lead to 6-carbon compounds very similar to carbohydrates.

Several experiments have tested the effect of ultraviolet light on glycolic acid. The irradiation was carried out in a solution containing hydrogen cyanide, ammonia, and a small amount of ferrous iron in addition to the glycolic acid. No attempt was made to attain a maximum yield of amino acids. Nevertheless hydrolysis of the nitriles formed revealed a yield of amino acids of 15 per cent, based on the original amount of glycolic acid. Examination of the products by paper chromatography produced a rather interesting result. Serine, the expected product, was



present, but alanine and glycine were also identified, as well as other substances. Furthermore, substantial quantities of more complex colored substances were formed. These were opaque to ultraviolet light and relatively easily adsorbed on talc. In nature, complex organic substances would be adsorbed and ultimately buried in sediments. Here is a possible mechanism for production of carbonaceous, organic sediments that does not invoke the action of living creatures.

Others who have speculated on the origin of life have postulated that a thick organic soup was formed and that when living creatures were available they quickly depleted this broth. Once the broth was gone the creatures had no alternative but to develop photosynthesis in order to survive. Consideration of the model that has been presented here does not lead to quite these same conclusions. If all the available hydrogen were used up in processes leading to formate, the maximum concentration of this substance would be 0.6 *M*; even allowing for the reducing capacity of sulfur and ferrous iron, the figure would rise to no more than 0.8 *M* as a maximum. The concentration of organic substances in the aqueous phase after a time would actually be considerably less as the longer, more complicated organic compounds were precipitated or adsorbed out of the system while relatively little of the diluting water was lost. It can be conceived that in the early stages of the earth as the oceans gradually grew in magnitude there was a relatively constant production of various kinds of organic molecules, some of which were lost by precipitation. Owing to this loss and to the continuing addition of water to the system, the concentration of organic molecules would actually diminish somewhat in spite of the constant rate of production. Under this picture the first living creatures which could extract organic compounds from the medium had available an annual supply of organic molecules, and hence there was not necessarily a catastrophic starvation period dur-

ing which creatures were forced to develop photosynthesis suddenly to survive. Rather a sharp competition for a limited food supply favored those creatures that could develop alternative energy sources such as photosynthesis.

One of the products of this research was an observation of the effect of ultraviolet light on ferrous iron. Many who have considered Precambrian geology have cited the widespread occurrence of ferric iron as evidence that in those times an oxygen atmosphere was present. Recent experiments have brought this assumption into question. Dilute ferrous iron at about *pH* 8 was placed in a silica flask, which was subsequently evacuated and subjected to ultraviolet light of wavelength 2536 Å. Hydrogen was formed, and a precipitate of ferric iron noted. Search of the literature then revealed that similar effects of ultraviolet light on ferrous iron had been demonstrated by Chastaing in 1877 and that a threshold of 2900 Å had been established for the reaction.

Others have pointed out that ozone which today absorbs ultraviolet light of wavelengths shorter than 3000 Å probably did not appear in quantity until living creatures invented photosynthesis. Comparatively large amounts of energy in the band 2540 to 2900 Å were hence available at the surface to act on the new ferrous iron released by weathering each year. No claim is made that these experiments prove that ferric iron in the Precambrian was due to the action of ultraviolet. On the other hand, it seems clear that the presence of ferric iron is not necessarily proof that either oxygen or photosynthesizing organisms were present at the time the ferric iron was laid down.

The result of this experiment with iron suggested still another, employing a solution of sodium sulfite at *pH* 8 together with a small amount of ferrous iron. Again the solution was placed in a silica vessel, which was evacuated and irradiated with 2536 Å ultraviolet light. Sulfate appeared in the solution. It would seem that the

occurrence of sulfate in the Precambrian is not necessarily diagnostic of oxygen in the atmosphere.

#### THERMAL DEGRADATION OF AMINO ACIDS

*J. R. Vallentyne*

Approximately  $10^{17}$  g of organic matter is synthesized annually on the earth's surface by plants. Although most of this is recycled in the biosphere, a small fraction becomes buried in sediments and soils, thus leaving the biochemically active part of the biosphere. Once sedimented, this organic matter is subjected to further decomposition, dependent on subsequent biological and chemical attack. If the fossil compounds occur in materials that are protected from biological attack (such as a calcite shell), then to a first approximation the system could be treated in terms of chemical kinetics. It is now well known (Year Book 53, p. 99) that amino acids occur in fossils as old as the Devonian. It is also known that certain amino acids are geologically less stable than others, for example serine, threonine, and phenylalanine as compared with the more stable glutamic acid, glycine, and alanine.

From a knowledge of the relative decomposition rates of amino acids under defined conditions in the laboratory, coupled with analyses of fossil materials, geological temperatures of preservation might be inferred if age is known. Two difficulties of interpretation should be clearly stated, however. In the first place, the medium of preservation (for example, calcite, bone, or shale) must be expected to influence the decomposition rates of amino acids. For the exact interpretation of data from fossil materials kinetic experiments must be conducted in media that simulate their geological counterparts. For example, the decomposition rates of amino acids in modern shells could be determined by analyzing for amino acids before and after a given treatment. Second, since kinetic experiments are limited in duration to a few years at the most, a high degree of

extrapolation is required to obtain estimates of decomposition rates at low temperatures. Extrapolation is always dangerous. Extremely sensitive methods for the analysis of decomposition products aid in extending experimental values to low temperatures, but do not overcome the difficulty completely. We will probably have to rely on the type of argument used by geochronologists in measuring age: if two independent measurements lead to a prediction of a single age, that value is more reliable than the same value determined by either method alone. Thus, if a temperature determination based on amino acids agrees with another based on a different set of compounds, a qualitative feeling of confidence results.

A preliminary attack on some of these problems has been made by studying the decomposition rates of amino acids in dilute aqueous solution. These data will serve as a base line with which future data (with the medium as the variable) can be compared. Decomposition rates for a variety of reactions can be expressed in terms of the Arrhenius equation:

$$k = se^{-\Delta H_a/RT}$$

The specific reaction rate constant,  $k$ , is the reciprocal of  $t_{1/e}$ , where  $t_{1/e}$  is the time (in seconds) required to decompose 63 per cent of the initial amount of the compound, at a given temperature. The frequency factor,  $s$ , depends on the type of reaction. It has a value of  $10^{13} \text{ sec}^{-1}$  for unimolecular reactions and much lower values for bimolecular reactions. In logarithmic form the Arrhenius equation is:

$$\log t_{1/e} = \Delta H_a/2.303RT - \log s$$

An increase in  $\Delta H_a$ , the activation energy, will thus markedly increase  $t_{1/e}$ , but an increase in  $s$  leads to a decrease in  $t_{1/e}$ . For  $\alpha$ -alanine (Year Book 53, p. 101),

$$k = 3 \times 10^{13} e^{-44,000/RT}$$

The comparable equation for phenylalanine (0.002 *M* solution) is:

$$k = 1.7 \times 10^8 e^{-30,800/RT}$$



with both sets of data shown graphically in figure 18. At temperatures below  $280^{\circ}\text{C}$ , phenylalanine decomposes more quickly than alanine under the same conditions. It can be predicted that after 5 to 10 million years' storage at  $30^{\circ}\text{C}$  most of the phenylalanine will have been degraded.

portance of extending the approach to other amino acids.

That water is involved in the decomposition of phenylalanine under the conditions employed is evidenced by the fact that the decomposition follows first-order kinetics over the concentration range  $2 \times 10^{-4} M$  to

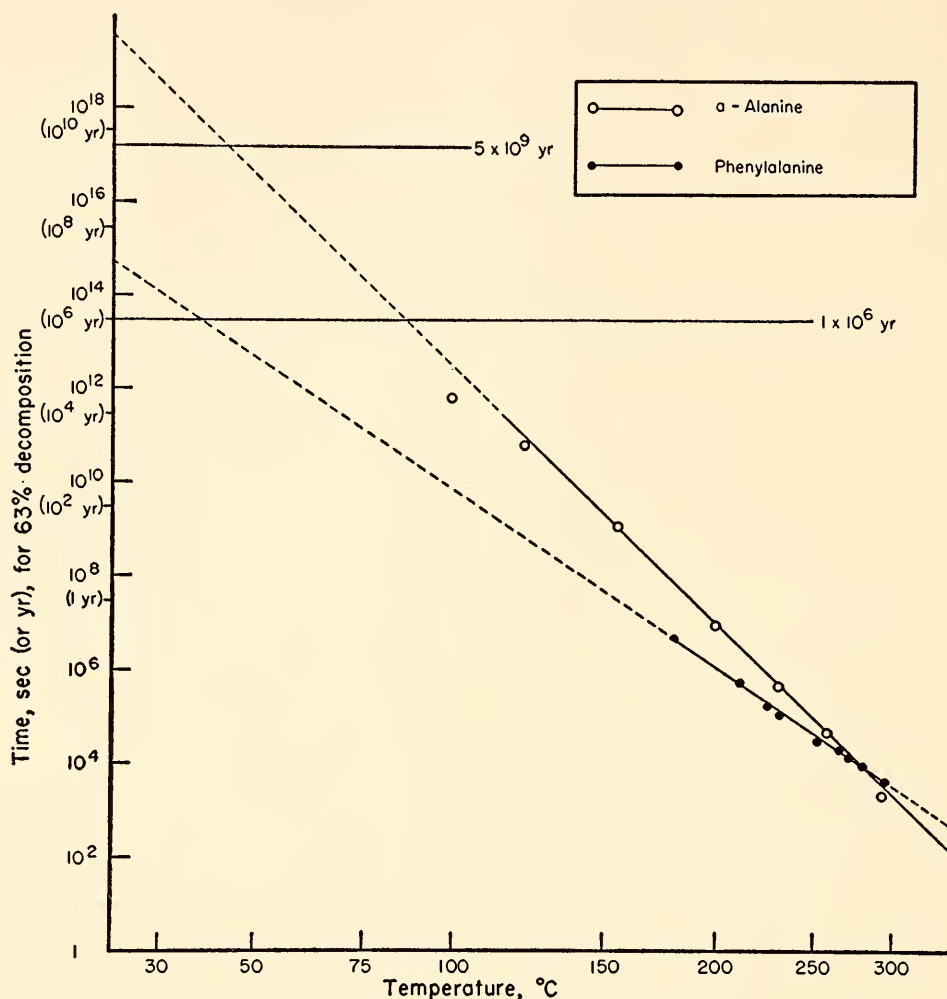


Fig. 18. Time required for 63 per cent decomposition of  $\alpha$ -alanine and phenylalanine (in dilute aqueous solution) at various temperatures. The scale of the horizontal axis is in units of the reciprocal of the absolute temperature.

Abelson (Year Book 53, p. 101) has noted that recent shells of the clam *Mercenaria mercenaria* contain both alanine and phenylalanine, whereas in Miocene shells of the same species alanine is present but phenylalanine is lacking. This qualitative agreement between analytical and (extrapolated) experimental data suggests the im-

portance of extending the approach to other amino acids. That water is involved in the decomposition of phenylalanine under the conditions employed is evidenced by the fact that the decomposition follows first-order kinetics over the concentration range  $2 \times 10^{-4} M$  to  $4 \times 10^{-3} M$ , with the value of the specific reaction rate constant,  $k$ , independent of concentration. Only the Arrhenius equation reveals that the reaction is bimolecular. Since water is involved in the decomposition of phenylalanine, it may be important to record the water contents of fossil materials before analysis.

## ORE MINERALS

Results important to our understanding of mineral associations found in nature have been obtained during this past year

from systematic laboratory studies of the subsolidus relations among some of the more common ore minerals. The investi-

gations of the stability relations of the most common sulfide, pyrite, and of the important ore mineral covellite have been completed. Studies of the composition of pyrrhotites formed in equilibrium with pyrite at various temperatures and pressures are near completion. In addition, the phases and solid solutions occurring in the  $\text{CoAs}_2$ - $\text{NiAs}_2$ - $\text{FeAs}_2$ -As system, as well as those in the Fe-S-O system, are nearly finished. Studies are also progressing on the Cu-Fe-S, Fe-Ni-S, Fe-Zn-S, and Fe-S-Se systems.

Some of the geological thermometers based on sulfide assemblages appear to be well established as useful tools. The FeS-ZnS system has been employed in measurements on more than a hundred ore deposits. As studies of more systems are completed in the laboratory, it should be possible to obtain cross checks between temperatures determined by observations of different assemblages occurring in the same deposit.

Detailed studies of ore deposits, aided by such laboratory tools, may in the future materially assist the field investigator in his interpretation of mineral associations and textures and will enable him to determine temperature, and possibly pressure, of formation of a host of mineral assemblages. These data are essential if attempts are to be made to estimate the composition of the solutions that transported the ore minerals to their site of deposition and to determine the direction of movement of these solutions.

#### THE Fe-S SYSTEM

*Stability relations of pyrite* (Kullerud, Yoder). The upper stability limits of pyrite,  $\text{FeS}_2$ , have been carefully determined, and the role of this binary compound in the Fe-S system can now be specified. Interpretation of the significance of the existence of an invariant point in which the phases pyrrhotite, pyrite, liquid, and vapor are stable unites many seemingly unrelated experiments. For example, previous work at pressures less than 1 atm has been related to new studies at high

pressures. A critical theoretical evaluation of the  $P$ - $T$ - $X$  diagram of the system Fe-S has brought forth the advantages and limitations of the various types of experimental techniques employed. These theoretical deductions and experimental techniques, some applied for the first time to sulfides, permit a new understanding of many of the ore-forming mineral assemblages.

The revised upper stability curve of pyrite,  $\text{FeS}_2 \rightleftharpoons \text{Fe}_{1-x}\text{S} + L$ , is given in figure

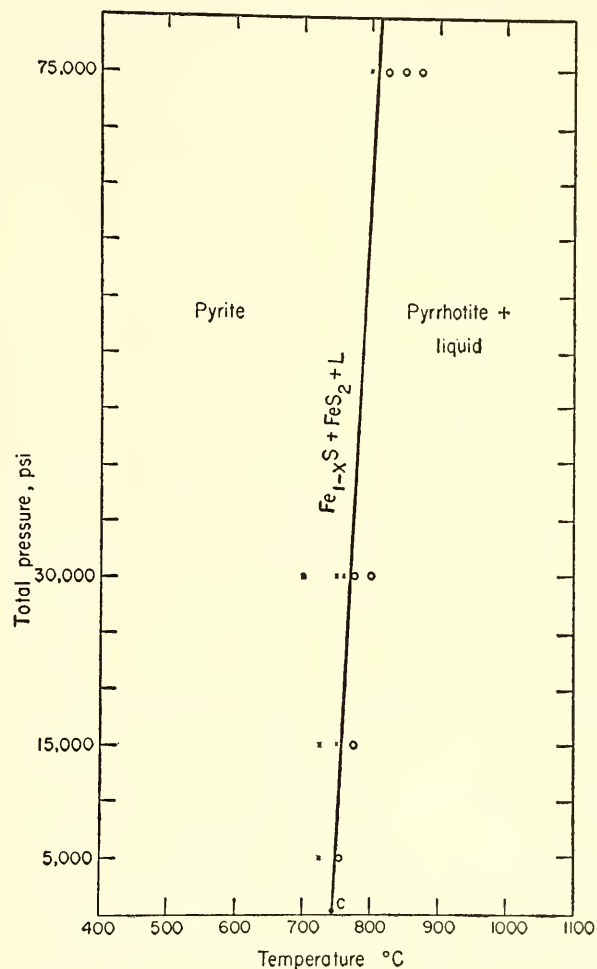


Fig. 19. Revised upper stability curve of pyrite,  $\text{FeS}_2 \rightleftharpoons \text{Fe}_{1-x}\text{S} + L$ .

19. It is to be noted that the reaction does not involve a gas phase. The experimentally determined points are  $815^\circ \text{C}$ , 75,000 psi;  $770^\circ \text{C}$ , 30,000 psi;  $755^\circ \text{C}$ , 15,000 psi;  $748^\circ \text{C}$ , 5000 psi. The curve terminates at the invariant point  $c$ , which lies at  $743^\circ \pm 3^\circ \text{C}$  and about 180 psi. All these points, with the exception of  $c$ , were determined using collapsible gold tubes (see Year Book 55, p. 181). Point  $c$ , where the four phases pyrrhotite, pyrite, liquid, and vapor



coexist, was determined in evacuated, rigid, silica-glass tubes. Since some of the experimental methods restrict the possible products, it was necessary to consider the relation of these products to the Fe-S system.

In figure 20 is given the schematic pressure-temperature diagram for the Fe-S system as deduced from published and new data using principles based on Gibbs' phase rule. Because of the large pressure range, only a schematic diagram is possible, although an attempt was made to maintain the temperature scale where pos-

“the minimum temperature of liquefaction,” of the primitive system Fe-S. The four univariant curves originating from *a* can be deduced by means of the principles outlined by Morey and Williamson (1918). One of these, the curve labeled  $L+V+S$ , terminates at the invariant point *b*, which has been found by experiment to lie at 115° C and 0.018 mm Hg (Tuller, 1954). Point *b* is the triple point of monoclinic sulfur. Portions of the curves  $L+S$  and  $\bar{V}+S$  have been investigated previously, and the curve  $L+V$ , which terminates at

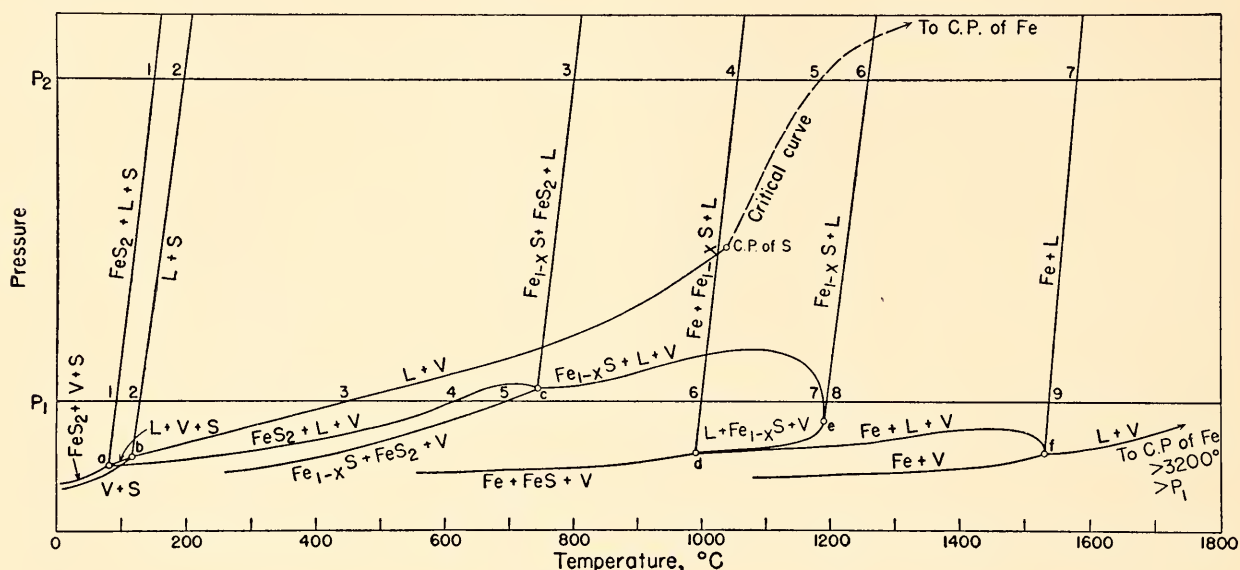


Fig. 20. Schematic pressure-temperature diagram for the Fe-S system. The pressure  $P_1$  is approximately 1 atm;  $P_2$ , several thousand atmospheres.

sible. The pressure  $P_1$  is approximately 1 atm, and  $P_2$  may be considered equal to several thousand atmospheres. Temperature-composition sections at these two pressures are given below. The details of construction of the  $P$ - $T$  diagram are presented in order of increasing temperature. For clarity of presentation the polymorphic phase changes have been neglected. The phases  $\text{Fe}_2\text{S}_3$  and  $\text{Fe}_3\text{S}_4$  (smythite) have also been neglected because their stability fields, if any, are not known with certainty.

The invariant point *a* is the temperature and pressure at which the four phases<sup>1</sup>  $\text{FeS}_2 + L + V + S$  coexist; it is the eutectic,

<sup>1</sup> The phases are arranged in the order of their composition, from the most iron-rich first to the most sulfur-rich last.

a critical point, has been estimated. The critical point of sulfur is believed to be about 1040° C and approximately 116 atm, and the boiling point (1 atm) is 444.6° C, according to West (1950). The curve  $\text{FeS}_2 + L + V$ , for which few experimental data are known to the writers, terminates at the point *c*, 743° C and about 180 psi. The pressure of point *c* was ascertained by extrapolating the data on the curve  $\text{Fe}_{1-x}\text{S} + \text{FeS}_2 + V$  given by Allen and Lombard (1917) to 743° C, the temperature determined by the present writers for the breakdown of pyrite in the presence of vapor. Some data for the remaining three curves originating at *c* have been obtained. The curve  $\text{Fe}_{1-x}\text{S} + \text{FeS}_2 + L$  is that given in figure 19. Allen and Lombard (1917) as

well as others give data on the curve  $\text{Fe}_{1-x}\text{S} + \text{FeS}_2 + V$  in the region of about 1 to 680 mm Hg. They indicate that this curve probably reaches 1 atm at about  $689^\circ\text{C}$ . No data are available for the solubility curve  $\text{Fe}_{1-x}\text{S} + L + V$  except in the vicinity of  $e$ . The continuation of this curve,  $L + \text{Fe}_{1-x}\text{S} + V$ , now a decomposition curve, terminates at  $d$ . The phases remain the same; the liquid becomes more iron rich than the crystalline phase, however.

The point  $e$  was carefully investigated by Jensen (1942); it marks the congruent melting of the binary compound  $\text{Fe}_{1-x}\text{S}$ . The temperature is given as  $1190^\circ\text{C}$ ; moreover, the pressure, which was not determined, is that of the vapor of the system. Jensen also determined the curve  $L + \text{Fe}_{1-x}\text{S} + V$  and the invariant point  $d$  where  $\text{Fe}$ ,  $L$ ,  $\text{Fe}_{1-x}\text{S}$ , and  $V$  are in equilibrium. Point  $d$  is given as  $988^\circ\text{C}$ , and the pressure, not determined, is that of the system. No data are known for the curves  $\text{Fe}_{1-x}\text{S} + L$ ,  $\text{Fe} + \text{Fe}_{1-x}\text{S} + L$ , or  $\text{Fe} + \text{Fe}_{1-x}\text{S} + V$ . The curve  $\text{Fe} + L + V$  has been studied by Friedrich (1908, 1910) and others. The point  $f$  is the triple point of iron. The melting point of iron under 1 atm of helium is  $1539^\circ\text{C}$ , according to Roeser and Wensel (1942). The vapor-pressure curve of pure iron,  $\text{Fe} + V$ , has been calculated by Jones, Langmuir, and MacKay (1927), and the vapor pressure at the melting temperature is estimated to be about 0.03 mm Hg. On the basis of these data the point  $f$  lies near  $1539^\circ\text{C}$  and 0.03 mm Hg. The same authors give the boiling point of iron as about  $3202^\circ\text{C}$ , and the critical point, therefore, must lie at a temperature greater than  $3202^\circ\text{C}$  and at a pressure in excess of 1 atm. The critical point of iron, not indicated in figure 20, is joined by the critical curve (dashed) to the critical point of sulfur.

Two isobaric sections through the  $P$ - $T$  diagram are presented in figures 21 and 22 for pressures  $P_1$  and  $P_2$ , respectively. The  $T$ - $X$  diagram of figure 21 is believed to represent the equilibrium relations that would be observed at a pressure of about

1 atm. The numbered points on the  $P$ - $T$  diagram (fig. 20) along the line  $P_1$  may be located on the  $T$ - $X$  diagram (fig. 21). Similarly the schematic  $T$ - $X$  diagram for  $P_2$  is believed to represent the relations at a pressure of several thousand atmospheres. These *sections* differ from the customary *projection* of the so-called "condensed"

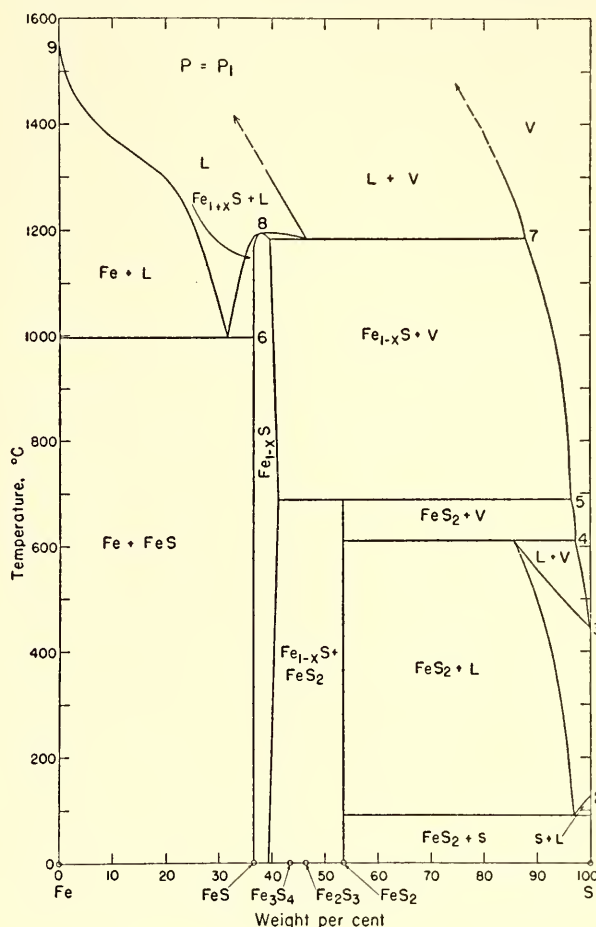


Fig. 21. The  $T$ - $X$  diagram for the  $\text{Fe}$ - $\text{S}$  system at the pressure  $P_1$ , approximately 1 atm (see fig. 20).

diagram (see, for example, Ricci, 1951, p. 63) given in figure 23, wherein the system is under its own pressure and gives those phases in equilibrium with vapor. The term "condensed" is, therefore, a misnomer, especially in systems containing volatile components, since vapor is present even though it is neglected. A truly condensed diagram would be one from which vapor (or gas) is absent for all assemblages. Such a  $T$ - $X$  diagram at constant  $P$  is not possible for the  $\text{Fe}$ - $\text{S}$  system. The diagram of figure 23 is produced by pro-



jecting onto the  $T$ - $X$  plane those curves in figure 20 that contain a vapor phase. The pressure is not constant, and is fixed only when two phases in addition to vapor are present as given by the three-phase curves. When only one or two phases are present the pressure is indeterminate unless the volume of the system is specified.

With the aid of these diagrams the na-

is limited by the strength of the heated silica-glass tube. The tube may be supported by an external pressure in some applications in order to increase its usable pressure range. It should be emphasized that the rigid silica-glass-tube experiments, regardless of an external supporting pressure, cannot yield data for those reactions in which vapor is absent, as the curve in

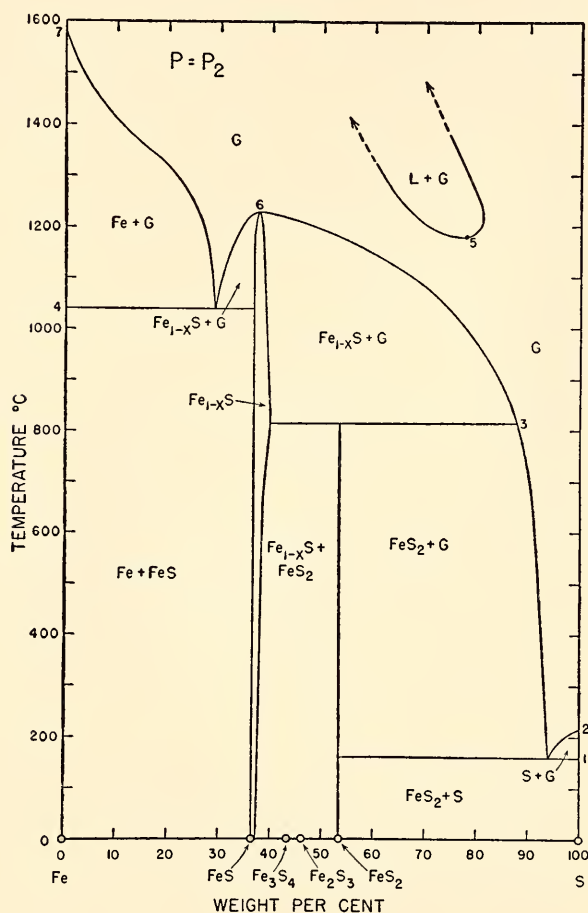


Fig. 22. The  $T$ - $X$  diagram for the Fe-S system at the pressure  $P_2$ , several thousand atmospheres (see fig. 20).

ture of the various types of experiment employed can now be elucidated. The evacuated rigid silica-glass tube is the container most commonly used for systems involving sulfur. Since the tube is rigid ( $\approx$ constant volume), and free space is available, a vapor phase is always present. For this reason the silica-glass tube can yield only data which pertain, for example, to a diagram of the type given in figure 23, the so-called condensed diagram. The pressure is usually unknown and, of course,

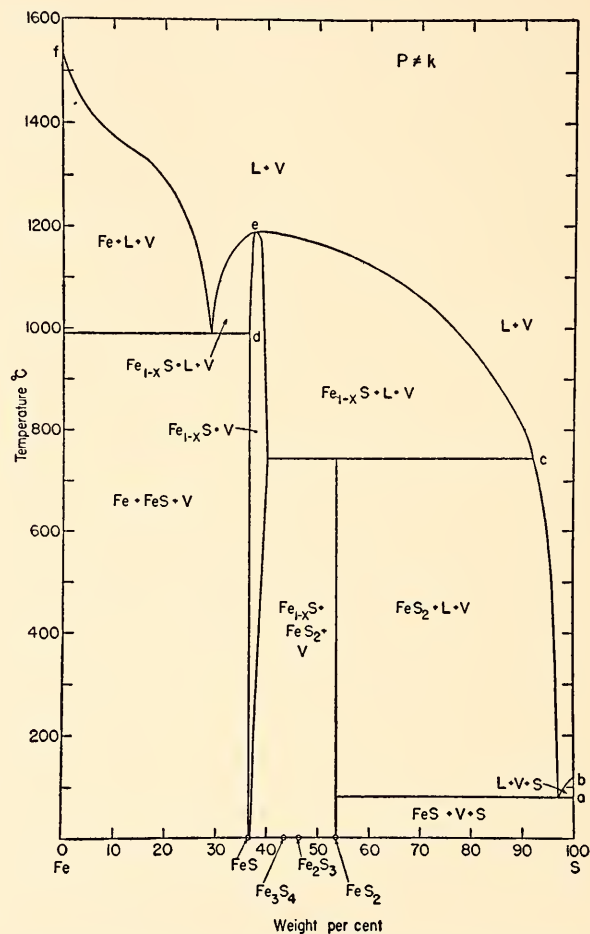


Fig. 23. The so-called "condensed" diagram of the system Fe-S. Vapor is present in all assemblages, and the pressure of the system is not constant.

figure 19 and the other essentially vertical curves in figure 20.

A second type of experiment employs collapsible gold tubes. Here the walls of the container are deformed by application of an external pressure: the internal pressure is taken to equal the external pressure. If the applied pressure is greater than the vapor pressure of the chemical system, then the vapor is condensed and no vapor is permissible. On the other hand,

if the applied pressure is less than the vapor pressure of the chemical system, then a vapor may exist. By this technique the entire  $P$ - $T$ - $X$  space may be investigated, yielding data both on the regions in which vapor is permitted and on those in which it is prohibited.

A third type of experiment employs two silica-glass bulbs connected by a tube. Sulfur is maintained at a given temperature in one bulb, and its vapor pressure is thereby fixed. In the other bulb the sulfur-containing system to be investigated is maintained at a series of temperatures. In this way the system in the latter bulb is held at a constant vapor pressure. Such experiments would yield data for those regions in figure 21, for example, where vapor is permitted. The regions in which vapor is prohibited (namely,  $\text{Fe} + \text{FeS}$ ,  $\text{Fe} + L$ ,  $\text{Fe}_{1-x}\text{S}$ ,  $\text{Fe}_{1-x}\text{S} + \text{FeS}_2$ ) could not be studied by this technique.

In a fourth technique the pressure of the system is measured by the deflection of the spiral of a spiral silica-glass-tube pressure gauge. Here again free space exists, and only those regions in which vapor is permitted can be studied.

Since the compositions of the coexisting sulfides in nature indicate that ore deposits may form in either the absence or the presence of a sulfur vapor (or gas), it is of paramount importance that the complete  $P$ - $T$ - $X$  space be investigated for the sulfide systems. These results in conjunction with hydrous systems will have important bearing on the problem of how metals are transported to the site of accumulation.

*The FeS-S join (Arnold).* The phase relations involving pyrrhotite, pyrite, liquid, and vapor are being studied between the compositions stoichiometric FeS and pure sulfur from 325° to 785° C. The relationship between pyrrhotite and pyrite, which is a portion of this system, promises to provide a method for estimating the temperature of formation of naturally occurring pyrrhotites and pyrite assemblages, a pressure of formation being assumed. The method is based on the fact that the

composition of pyrrhotite when coexisting in equilibrium with pyrite is a function of temperature and pressure. Two experimental methods are used for studying the equilibrium relations between the coexisting phases. The first employs silica-glass tubes as sample containers; as a result, a vapor exists at each temperature above the solid and/or liquid phases by virtue of the presence of a vapor space in each tube. The second method involves the use of collapsible gold tubes as sample containers. These two methods and the results obtained are discussed separately.

The equilibrium relations between pyrrhotite and pyrite were studied by the silica-tube method from 325° to 743° C at vapor pressures from a few millimeters of mercury to about 10 bars, respectively; pyrite and liquid were studied up to 743° C, where the vapor pressure was about 10 bars; and pyrrhotite and liquid were studied from 743° to 785° C at vapor pressures from 10 to 25 bars, respectively. The magnitude of the vapor pressure above pyrrhotite and pyrite at a specific temperature is estimated from the data of Allen and Lombard, De Rudder, D'Or, Roedder, Juza and Biltz, and Rosenqvist.

Equilibrium at each temperature was approached from two directions. All runs withdrawn from a furnace were immediately quenched in cold water. Temperature measurements were within 4° C unless otherwise indicated. Pyrrhotite compositions were determined to a precision of  $\pm 0.08$  atomic per cent iron by the lattice-spacing technique described in last year's report. Pyrite, as pointed out in that publication, shows no measurable variation in its cell dimensions at a considerable range of temperatures, indicating little or no variation in its metal-to-sulfur ratio. Figure 24 is a diagram for a condensed system at 30 bars pressure (refer to the discussion by Greig, Year Book 54, p. 131), which summarizes the equilibrium relations between pyrrhotite, pyrite, and liquid as calculated on the basis of this study and by the work of Jensen. Although the ex-



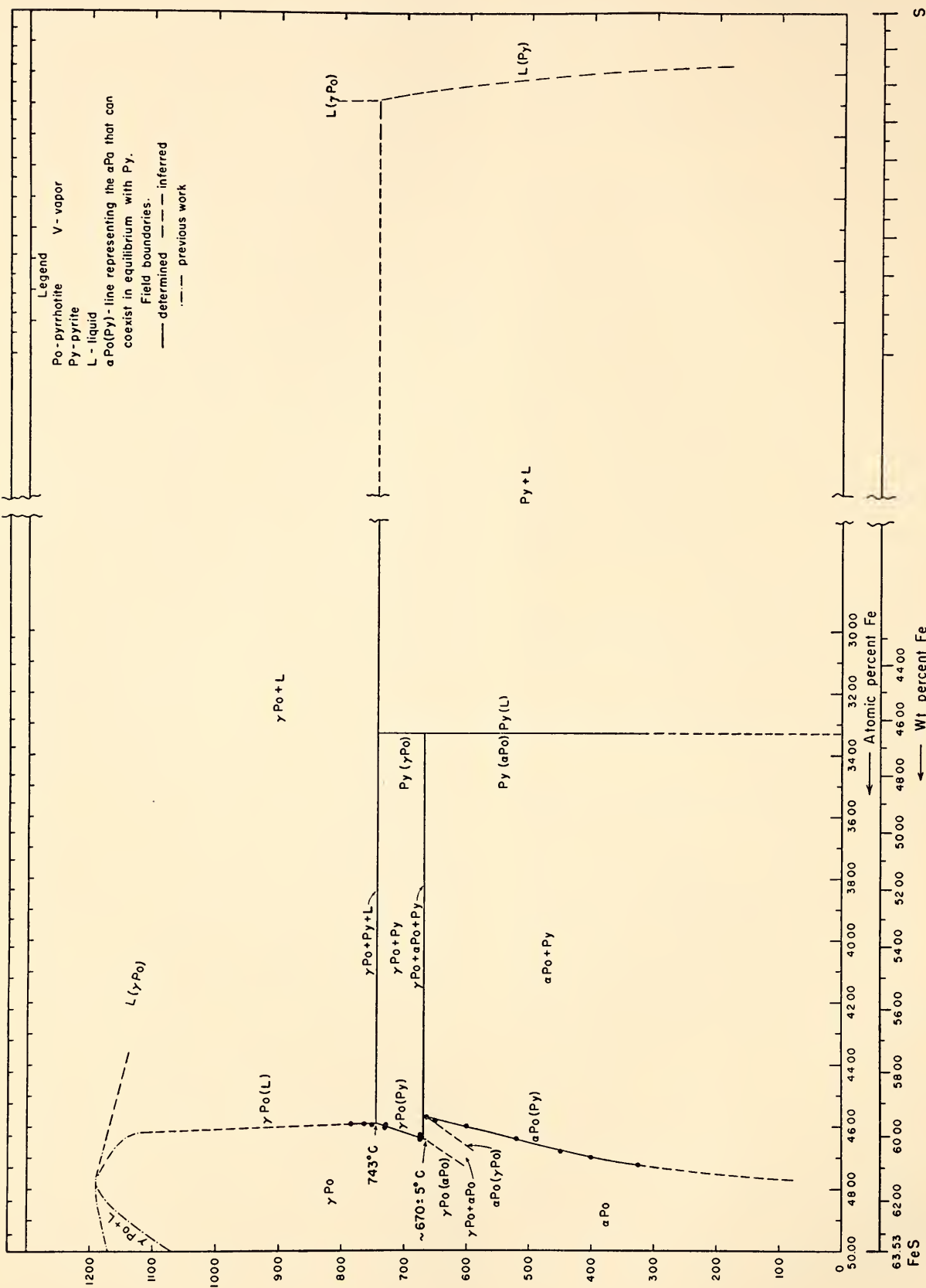


Fig. 24. Tentative FeS-S equilibrium diagram for a pressure of 30 bars. Data points represent pyrrothite compositions projected on this isobaric section.

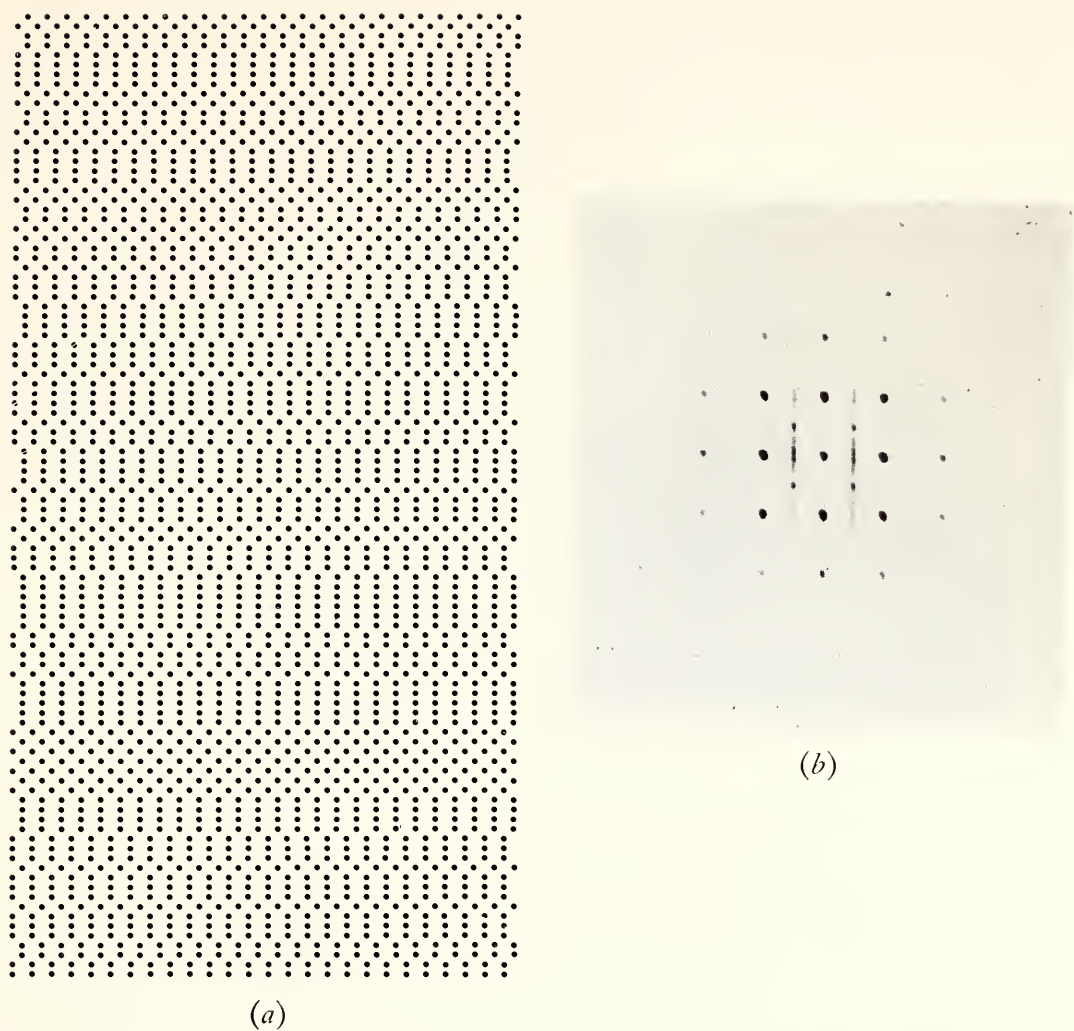


Fig. 2. "Layers" of equal scattering power with displacement of  $b/2$  randomly distributed; equal numbers of "layers" in each of the two positions.  $a$ , the mask;  $b$ , diffraction pattern of  $2a$ .

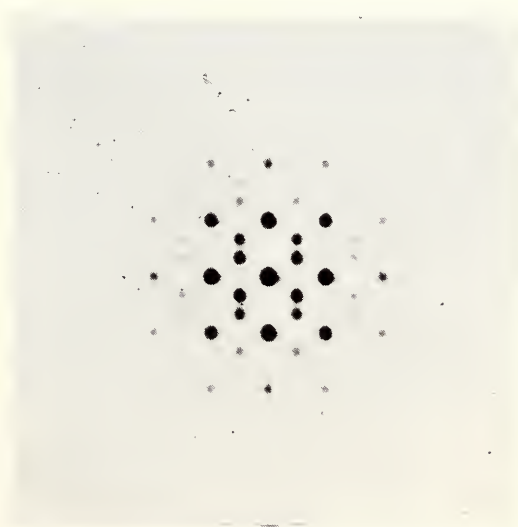
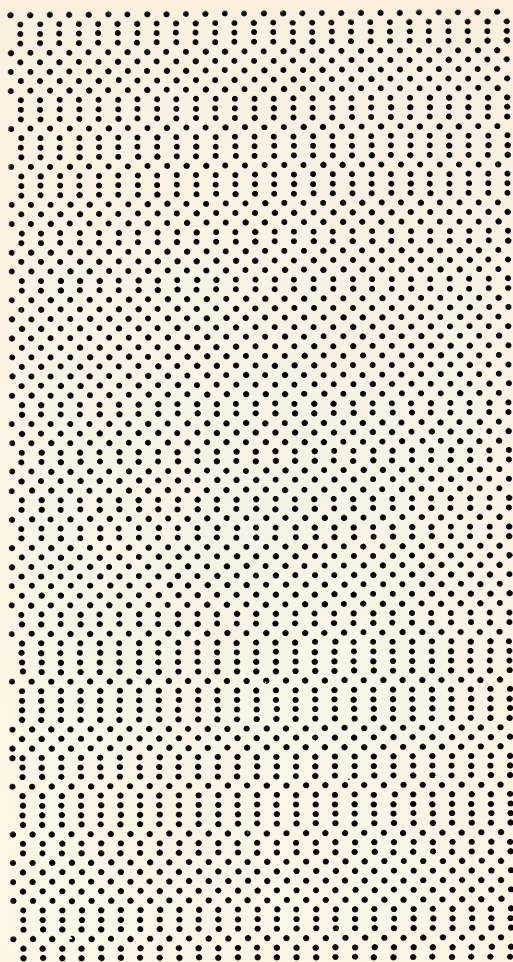
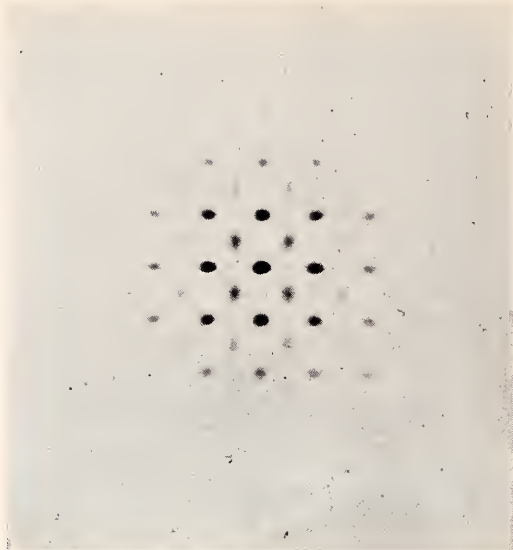


Fig. 3. Diffraction pattern of a perfectly ordered sequence of two lines in one position followed by one line in the other ( $BBABBABBA \dots$ ).

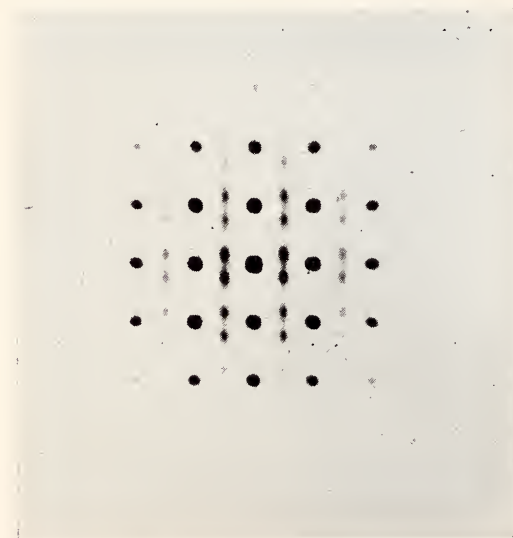




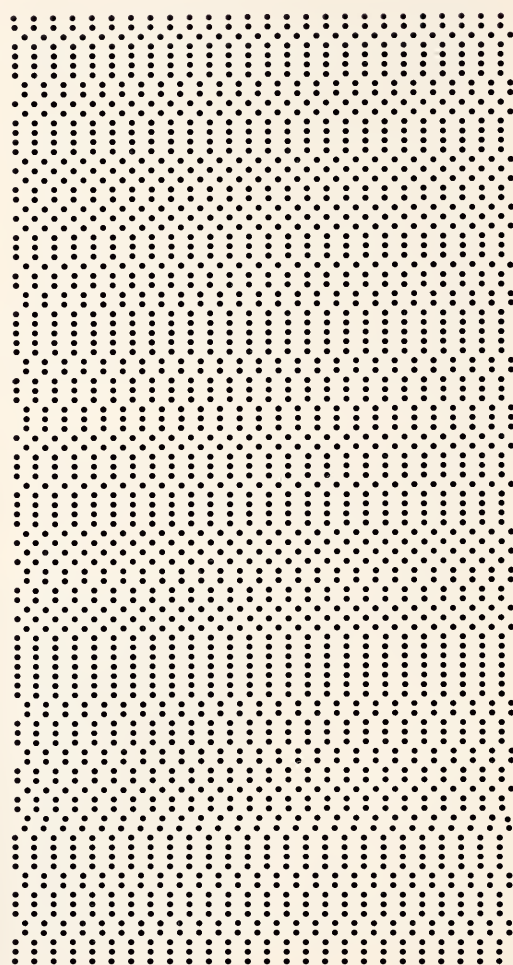
4 (a)



4 (b)



5 (b)



5 (a)

Fig. 4. Short-range ordering in a "2:1" mask: *a*, a mask in which  $N_B = 2N_A$ , each *A* is neighbored by *B*'s but the run lengths of *B*'s are random; *b*, diffraction pattern of 4*a*.

Fig. 5. Disordered "2:1" mask: *a*, a mask in which  $N_B = 2N_A$ , but run lengths and sequences of both *A* and *B* are random; *b*, diffraction pattern of 5*a*.

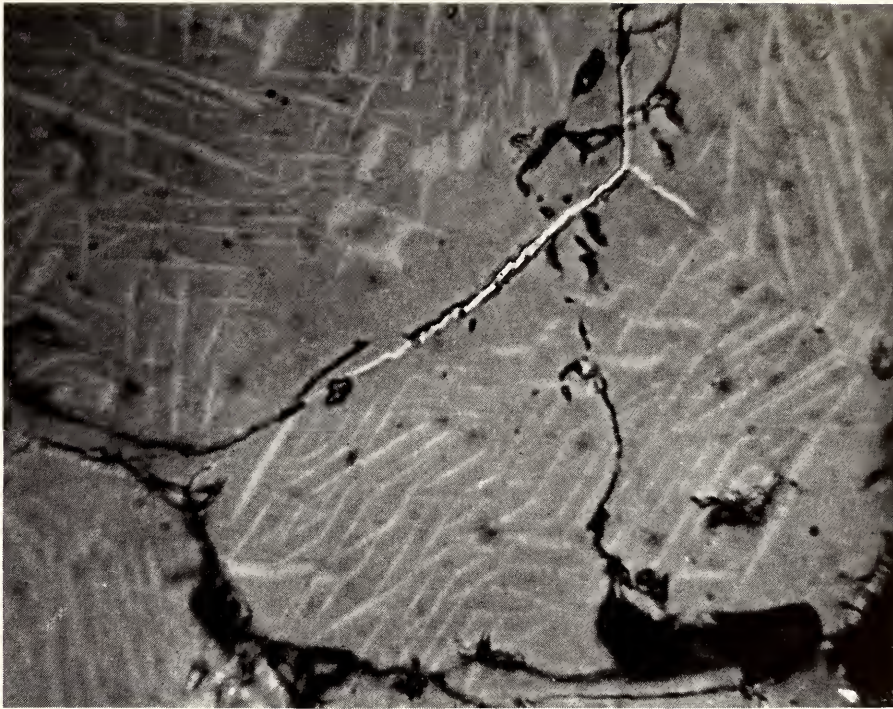


Fig. 25. Photograph showing exsolution lamellae in pure pyrrhotite. Slight granularity due to the second dark phase may be seen in the host. The veinlet in the center of the photograph is pyrite.  $\times 1200$ .





periments were made at various pressures lower than 30 bars, it is practicable to construct a section at this pressure, because the effect of pressure on the melting points, field boundaries, and inversion temperatures is comparatively small. For instance, a calculation based on the data presented by Kullerud and Yoder in last year's report shows that the melting point of pyrite at 30 bars is only about  $\frac{1}{2}^{\circ}$  C higher than at about 10 bars. Also by calculation based on the data presented in figure 27 the position of the field boundary  $\alpha\text{Po}(\text{Py})$  at 30 bars is shifted only 0.006 atomic per cent iron towards the iron side of the diagram.

The supercell and monoclinic forms of pyrrhotite stable below  $138^{\circ}$  C discussed by Haraldsen have been omitted from the diagram because the relationships in this area are not understood. Also marcasite ( $\text{FeS}_2$ ) and smythite ( $\text{Fe}_3\text{S}_4$ ) have not been included, as their fields of stability are not known. The melting relations of pyrrhotite were determined by Jensen. The datum points plotted on the diagram represent compositions determined from the silica-tube runs projected on this isobaric section.

The notation suggested by Greig for designating the fields, field boundaries, and isothermal lines representing the conditions under which one or more phases exist in equilibrium has been found to be very useful, as the relations between the various phases become immediately obvious. For example,  $\alpha\text{Po}(\text{Py})$  designates the line indicating the conditions under which  $\alpha$ -pyrrhotite may coexist in equilibrium with pyrite;  $\text{Py}(\alpha\text{Po})$  designates the conjugate line indicating the conditions under which pyrite can coexist in equilibrium with  $\alpha$ -pyrrhotite.

As is indicated in figure 24, pyrite melts incongruently at  $743^{\circ}$  C to pyrrhotite having a composition of 45.95 atomic per cent iron and a liquid close to pure sulfur in composition. The compositions of the liquid that can coexist with pyrrhotite and of the liquids that can coexist with pyrite

have not been determined. The arrangement for the liquidus curves shown diagrammatically, however, is correct for the case that pyrite melts incongruently.

An inversion in the most sulfur-rich pyrrhotites was observed between  $666^{\circ}$  and  $675^{\circ}$  C in four runs; it is tentatively placed at  $670^{\circ} \pm 5^{\circ}$  C. The inversion temperature is believed to decrease with increasing iron content in pyrrhotite. The low-temperature form designated  $\alpha$ -pyrrhotite is quenchable and has the normal NiAs-type structure at room temperature. The high-temperature form designated  $\gamma$ -pyrrhotite is apparently nonquenchable, and inverts to the  $\alpha$  form on quenching.

Preparations of pyrrhotite coexisting with pyrite, quenched from above the inversion temperature, frequently showed exsolution lamellae when examined under the microscope (fig. 25).<sup>1</sup> The lamellae have the following general characteristics: they generally possess straight parallel sides, but occasionally occur in irregular blebs, both up to several microns in width; in some grains they are arranged in three or four intersecting sets; they have a reflectivity higher than the host, but much lower than pyrite; they are softer than the host, and are anisotropic. Both the pyrrhotite host and the lamellae may contain a second separate phase having a lower reflectivity and a greater hardness than either the host or the lamellae. This phase appeared as dispersed black dots in both the lamellae and the host, being preferentially concentrated in the host and imparting a granular texture to it. Occasionally this phase in the host also appeared as very fine subparallel lines producing a crepe-paper-like textural effect. Pyrrhotite structures with similar physical characteristics have been described from natural occurrences by Ramdohr, by Schneiderhöhm and Van Der Veen, and by Scholtz.

Powder camera photographs of these exsolved pyrrhotites at room temperature show no additional reflections that can be

<sup>1</sup> Figure 25 is on plate 3.



attributed to a second phase with a different symmetry or composition. Insufficient data are available to explain the presence of the various phases or to decide whether the lamellae were exsolved as the  $\alpha$  or the  $\gamma$  form. Very likely this pyrrhotite exsolution is related to the inversion that takes place at  $670^\circ \pm 5^\circ \text{C}$ .

The presence of the inversion and related exsolution raises the question of the applicability of the lattice-spacing-composition curve for evaluating pyrrhotite compositions. Because the pyrrhotites on which the spacing curve was based were homogeneous, this curve is pertinent only to homogeneous pyrrhotite. Only compositions of homogeneous pyrrhotites were used as a basis for constructing the equilibrium diagram.

The pyrrhotite-pyrite equilibrium was also studied between  $325^\circ$  and  $600^\circ \text{C}$  at 1000 and 2000 bars pressure. The apparatus was the standard cold-seal bomb fed by flexible capillary tubing. Quenching was effected by plunging the hot bomb directly from the furnace into water while simultaneously releasing the pressure. All runs were made in collapsible gold tubes, which transmitted the water pressure, essentially hydrostatically, to the contained sulfides. Equilibrium was approached from two directions at each pressure and temperature. The results are shown in figure 26, together with the equilibrium results determined at  $<1 \text{ atm}$  included for comparison. The results show that pressures up to 2000 bars do not measurably alter the equilibrium relations between pyrrhotite and pyrite as determined under their own vapor pressure at  $325^\circ \text{C}$  and lower. However, at  $600^\circ \text{C}$  and 1000 and 2000 bars the equilibrium composition of pyrrhotite coexisting with pyrite was 46.18 and 46.46 atomic per cent iron, respectively, while at  $<1 \text{ atm}$  pressure at  $600^\circ \text{C}$  the pyrrhotite composition was 46.03 atomic per cent iron.

The fact that the composition of pyrrhotite when coexisting in equilibrium with pyrite is a function of temperature

and pressure raises the possibility of the application of these relations to problems of geological thermometry. In applying this "thermometer" in its present state of development, however, at least one difficulty should be kept in mind.

Because the experimental system contained only iron and sulfur, the effect of impurities on the equilibrium value of the metal-to-sulfur ratio of pyrrhotite should

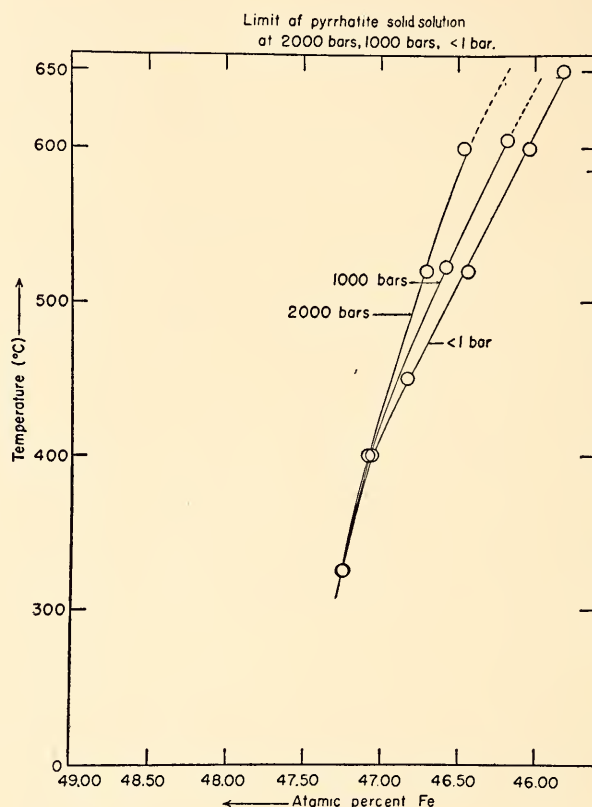


Fig. 26. Curves showing the effect of pressure of  $<1$ , 1000, and 2000 bars on the composition of pyrrhotite that can coexist in equilibrium with pyrite.

be investigated. Quantitative analyses of natural pyrrhotites indicate that cobalt and nickel are the most significant impurities, although rarely occurring in combined concentrations greater than 2 weight per cent.

An attempt was made to study the effect of 0.65 to 6.50 per cent cobalt on the equilibrium between pyrrhotite and pyrite at  $600^\circ \text{C}$ . It was hoped that the composition of the resulting cobalt-rich pyrrhotite coexisting in equilibrium with cobalt-rich pyrite could be determined by means

of an X-ray spacing technique. The curve determined at 730° C and intended for this purpose is given in figure 27. It shows the change of the pyrrhotite  $d(102)$  as various amounts of cobalt are substituted for iron in the stoichiometric pyrrhotite structure. In nonstoichiometric pyrrhotite, measurements indicated that a phenomenon additional to the replacement of iron by cobalt had taken place. Apparently a specific amount of cobalt as a function of pressure and temperature filled vacant iron positions in the nonstoichiometric pyrrhotite structure, producing a large increase in the  $d(102)$ ; additional cobalt replaced iron and caused a decrease in  $d(102)$  proportional to the amount available.

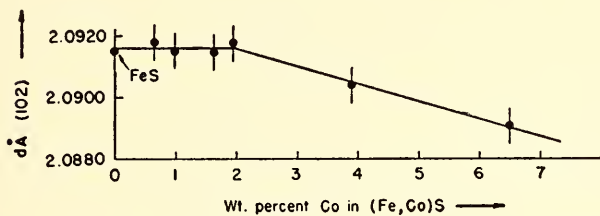


Fig. 27. Curve showing the effect of substituting cobalt for iron on the  $d(102)$  of stoichiometric FeS. Amounts of cobalt in excess of 1.95 weight per cent cause a decrease in the  $d(102)$ .

Because of this additional replacement effect, the proposed X-ray spacing technique could not be employed and the influence of cobalt on the equilibrium between pyrrhotite and pyrite could not be evaluated. Because nickel will no doubt produce similar problems, at this stage this thermometer should be applied only to deposits containing no more than small amounts of cobalt and nickel.

#### THE Cu-S SYSTEM

*The upper stability curve of covellite (Kullerud).* Covellite is one of the most important sulfides of copper. It occurs in association with other copper minerals such as chalcopyrite, chalcocite, or digenite. It is found in zones of secondary alteration, or as a primary mineral associated with chalcocite or digenite (Butte), and has even been reported as a sublimation product (Vesuvius). Knowledge of the sta-

bility field of covellite, therefore, would contribute to an understanding of the conditions existing during the formation of many copper-bearing sulfide ore deposits.

Covellite may be readily synthesized in the dry way in silica tubes by mixing copper with appropriate amounts of sulfur. Copper and sulfur in the atomic ratio of 1:1 will, in evacuated, sealed silica tubes, react even at 20° C to form some covellite in a few hours. Even after 5 months at this temperature, however, the tubes still contained small amounts of unreacted copper and sulfur. At 100° C all copper had reacted to form covellite in about 5 weeks, and at 200° C in about 2 weeks.

The lengths of the silica tubes were adjusted so that the copper-sulfur mixtures occupied a third to a half of the tube volume. It was noticed that at 235° C some digenite ( $\text{Cu}_9\text{S}_5$ ) was already formed with the covellite in the tubes. Thus covellite under such conditions starts to break down to digenite and sulfur vapor below 235° C. Sulfur vapor produced by this process builds up pressure to stabilize the remaining covellite. The dissociation pressures of covellite at temperatures ranging from 400° to 490° C were determined by Wasjuchnowa, by Preunner and Brockmoller, and by Allen and Lombard.

By adding sulfur beyond the 1:1 copper-to-sulfur ratio in the silica tubes so that liquid as well as vapor is always present, covellite was found to be stable up to  $507^\circ \pm 3^\circ$  C. Above this temperature digenite + liquid + vapor are stable. The pressure at the invariant point of  $507^\circ \pm 3^\circ$  C where  $\text{CuS} + \text{Cu}_9\text{S}_5 + L + V$  coexist may be determined by extrapolation of the  $P$ - $T$  curve given for dissociation of covellite by the above-mentioned workers. Such extrapolation gives a pressure of 880 mm Hg for the invariant point. At this point the following four curves intersect: (1)  $\text{Cu}_9\text{S}_5 + \text{CuS} + V$ ; (2)  $\text{CuS} + L + V$ ; (3)  $\text{Cu}_9\text{S}_5 + \text{CuS} + L$ ; and (4)  $\text{Cu}_9\text{S}_5 + L + V$ . The situation at the invariant point is shown schematically in figure 28. Curve 3,  $\text{Cu}_9\text{S}_5$



+CuS+L, is the upper stability curve of covellite. It was determined by using sealed collapsible gold tubes as described in last year's report for the determination of the upper stability curve of pyrite.

The upper stability curve of covellite is shown in figure 29. Points on the curve are: 507° C at about 880 mm Hg (invariant point *c*), 510° C at 7500 psi, 515° C at 15,000 psi, and 525° C at 30,000 psi.

Covellite below the invariant point *c* breaks down to digenite+vapor, not to chalcocite+vapor as reported by earlier workers. Above the invariant point *c*,

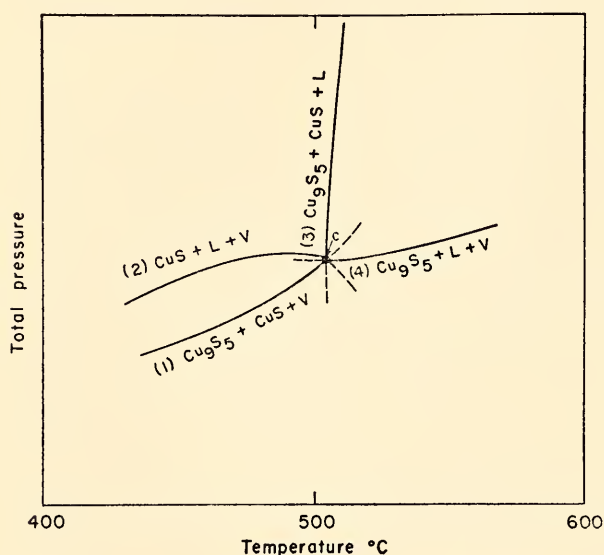


Fig. 28. Curves showing schematically invariant point *c* where the four phases  $\text{Cu}_9\text{S}_5 + \text{CuS} + \text{L} + \text{V}$  are stable.

covellite, in collapsible tubes, breaks down to digenite+liquid. In the diagram the stability field of covellite is on the left of the curve and the field of digenite+liquid is on the right.

Covellite grown in silica tubes is dark blue, is commonly massive, and rarely shows crystal faces. Single crystals were in a number of experiments grown at high pressure in collapsible gold tubes. These crystals, less than 0.1 mm long, appear to be hexagonal plates. Powder X-ray diffraction studies of covellite crystals grown under varied pressure and temperature conditions (from 100° C at less than 1 mm Hg to 520° C at 2000 bars) show no meas-

urable variations in the cell dimensions.

Digenite formed in these experiments by breakdown of covellite commonly grows as well developed crystals. They always occur as octahedra, which occasionally are extremely malformed and frequently modified by cube faces. Sometimes two opposite octahedral faces predominate over all

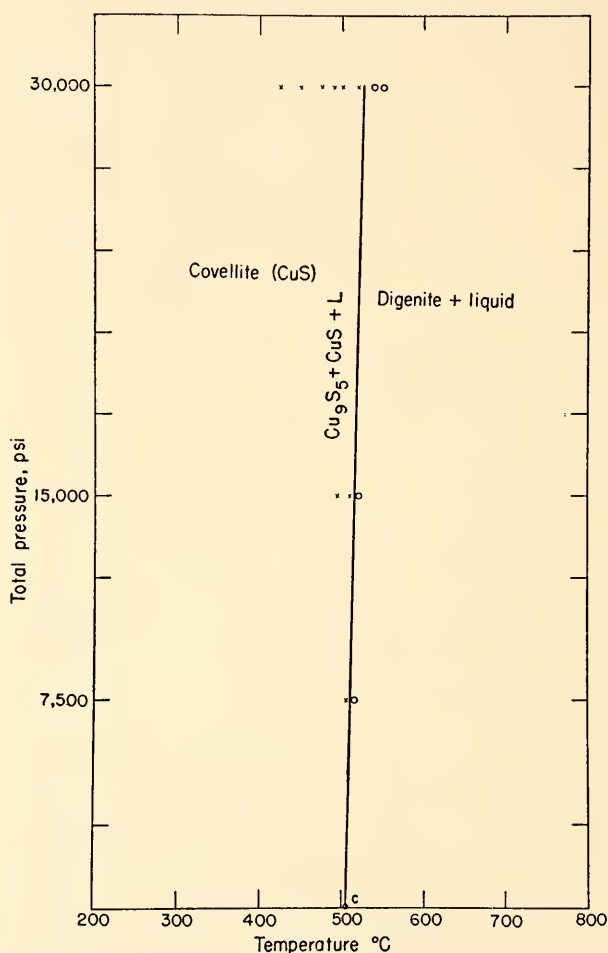


Fig. 29. The upper stability curve of covellite  $\text{CuS} \rightleftharpoons \text{Cu}_9\text{S}_5 + \text{L}$ .

other faces to such an extent that the crystal looks like a flat hexagonal plate. Other times, observed at 15,000 and 30,000 psi, cube faces dominate. The digenite crystals vary in size from about 0.1 to 4 mm, depending on time, temperature, and pressure. The larger crystals were grown in silica tubes, and the smaller in collapsed gold tubes. The cell dimensions of digenite formed at various temperatures and pressures were determined by X-ray diffraction methods. The cell size, however, remained constant within the limits of the

experimental accuracy, an indication that digenite is essentially a stoichiometric compound.

The liquid formed by decomposition of covellite when quenched appears white to yellow and consists of almost pure sulfur. X rays at room temperature of such quenched liquids, produced at various pressures and temperatures, always gave the pattern of orthorhombic sulfur.

crystals and  $\text{NiS}_2$  were described. A diagram was also presented showing variation in  $d$  spacings for the (102) X-ray reflections as a function of mix-crystal ( $\text{Ni}_{1-x}\text{S}$ ) composition. This curve was used to determine the composition of the  $\text{Ni}_{1-x}\text{S}$  when formed in equilibrium with  $\text{NiS}_2$ . Experiments with mixtures of Ni and S in the atomic ratio of 3:4 have established the subsolidus relations between the two

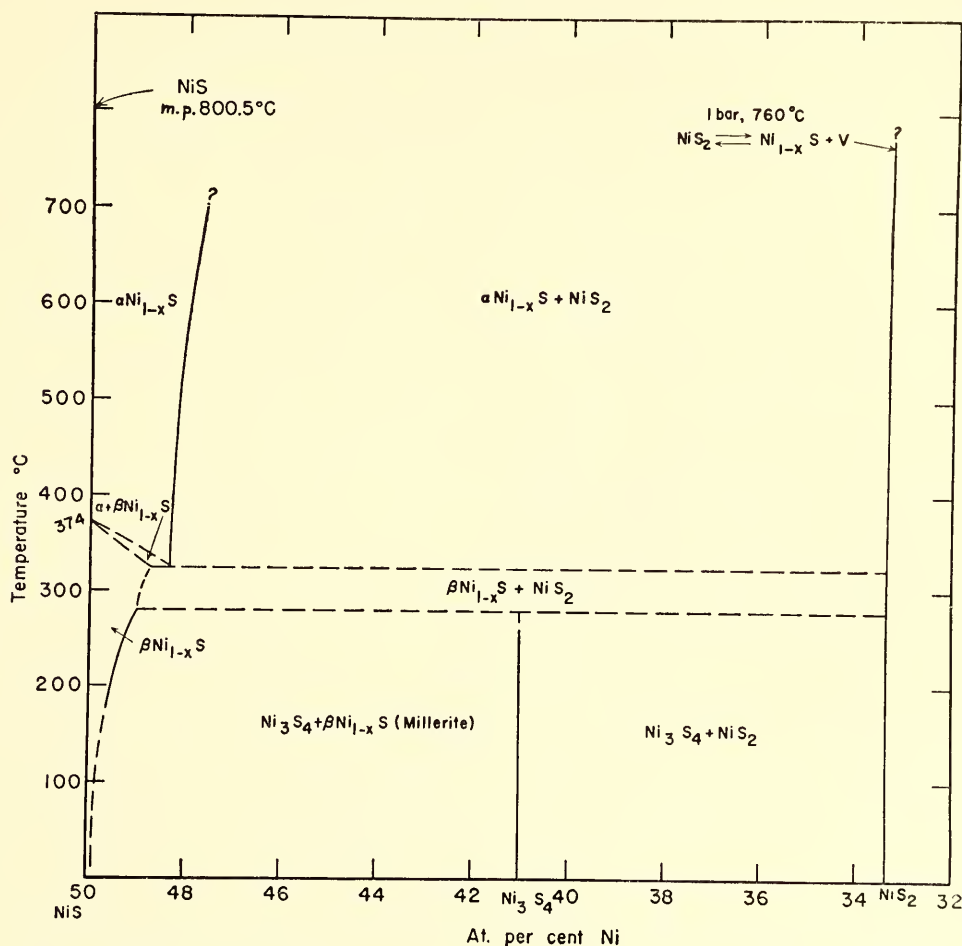


Fig. 30. Subsolidus phase relationships in the  $\text{NiS}$ - $\text{NiS}_2$  binary join.

#### THE Ni-S SYSTEM

*The NiS- $\text{NiS}_2$  join (Arnold, Kullerud).* This study of the subsolidus relations in the millerite ( $\text{NiS}$ )-vaesite ( $\text{NiS}_2$ ) binary join is part of an investigation of the entire Ni-S system. A systematic investigation of this system is necessary before a detailed exploration of the extremely important Fe-Ni-S system can be undertaken.

In last year's report methods of preparation of  $\text{NiS}$  as well as of  $\text{Ni}_{1-x}\text{S}$  mix-

phases in the 300° to 700° C temperature range. When stoichiometric  $\text{NiS}$  is heated a rapid inversion from the millerite to the hexagonal Ni-As structure occurs at 374° C. This inversion temperature is lowered markedly by omission of Ni from the  $\text{NiS}$  lattice. Thus when  $\text{Ni}_{1-x}\text{S}$  is formed in equilibrium with  $\text{NiS}_2$  the inversion takes place at about 325° C.

The  $\text{Ni}_3\text{S}_4$  phase, reported in the literature to be stable up to 325° C, did not appear at 300° C even after 6 months.



No sign of melting was detectable in  $\text{Ni}_{1-x}\text{S}$  and  $\text{NiS}_2$  mixtures at temperatures as high as  $790^\circ\text{C}$ , in spite of the fact that stoichiometric  $\text{NiS}$  melts at  $800.5 \pm 1^\circ\text{C}$ .

Figure 30 shows the phase relations in the  $\text{NiS}$ – $\text{NiS}_2$  join. The amount of nickel omission solid solution in  $\text{Ni}_{1-x}\text{S}$  when this phase forms in equilibrium with  $\text{NiS}_2$  is seen to vary from 1.7 atomic per cent

$V$  in silica tubes, as well as with  $\text{NiS} + \text{S}$  and  $\text{NiS}_2$  in collapsible gold tubes at 2000 bars, have shown that  $\text{NiS}_2$  under such conditions is stable beyond the melting point of  $\text{NiS}$  ( $800.5^\circ \pm 1^\circ\text{C}$ ). The conclusion is that the four phases  $\text{Ni}_{1-x}\text{S} + \text{NiS}_2 + L + V$  cannot coexist at any pressure or temperature.

Powder X-ray diffraction studies of  $\text{NiS}_2$

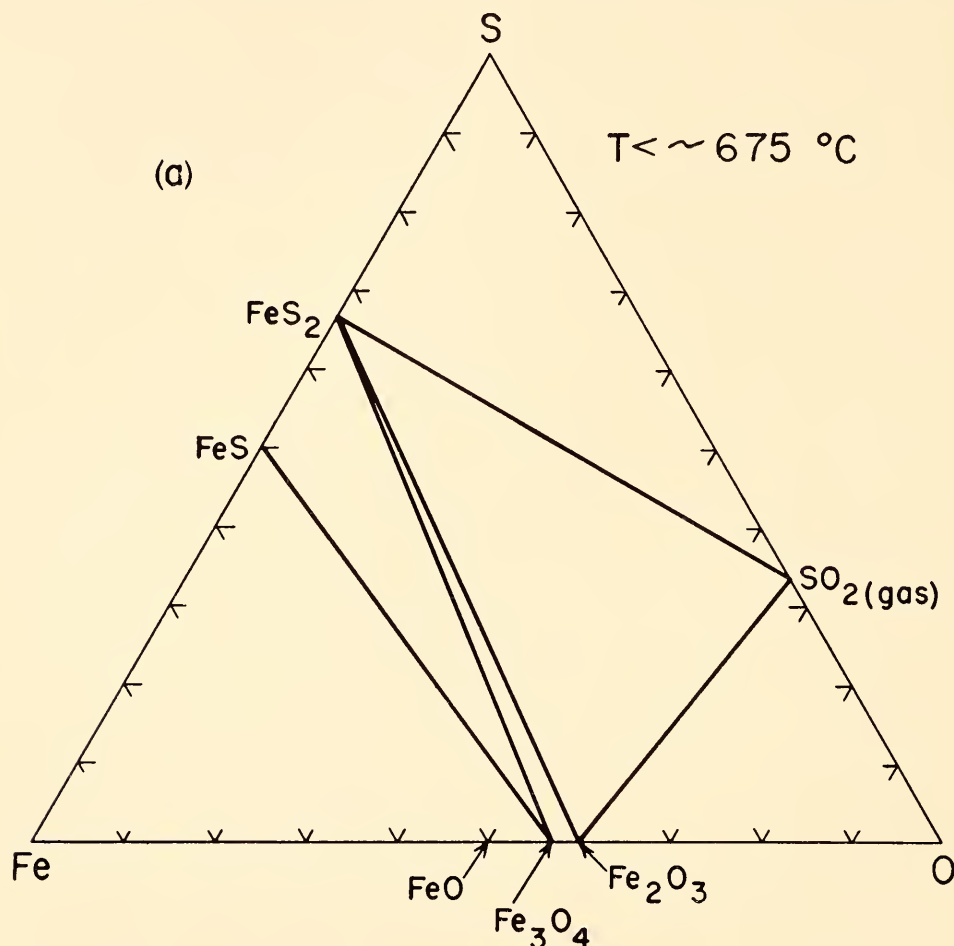


Fig. 31. Phase relations in the Fe–S–O system: (a) below about  $675^\circ\text{C}$ .

$\text{Ni}$  at  $325^\circ\text{C}$  to about 2.4 atomic per cent  $\text{Ni}$  at  $700^\circ\text{C}$ , giving rise to a very steep solvus curve.

Biltz (1936) determined the curve for dissociation of  $\text{NiS}_2$ : at  $650^\circ\text{C}$ , 42.5 mm Hg; at  $700^\circ\text{C}$ , 154 mm Hg; at  $720^\circ\text{C}$ , 260 mm Hg; at  $730^\circ\text{C}$ , 324 mm Hg; and at  $760^\circ\text{C}$ , 649 mm Hg.  $\text{NiS}_2$  in this range breaks down to  $\text{Ni}_{1-x}\text{S} + V$ . Extrapolation of these data gives breakdown of  $\text{NiS}_2$  under 1 atm sulfur pressure at  $765^\circ\text{C}$ . Preliminary experiments at  $850^\circ\text{C}$  with  $\text{NiS}_2$  with excess sulfur to give  $\text{NiS}_2 + L +$

grown at temperatures from  $350^\circ$  to  $854^\circ\text{C}$  and at pressures ranging from a few millimeters of mercury to 2000 bars show an apparent variation in the cell lengths of the  $\text{NiS}_2$  crystals from  $a = 5.685 \pm 0.002$  Å at  $350^\circ\text{C}$  and pressure of less than 1 mm Hg to  $a = 5.690 \pm 0.002$  Å at  $854^\circ\text{C}$  and 2000 bars.

#### PHASE RELATIONS IN THE Fe–S–O SYSTEM *G. Kullerud*

The Fe–S–O system includes pyrite ( $\text{FeS}_2$ ), the most common sulfide mineral,

and pyrrhotite ( $\text{Fe}_{1-x}\text{S}$ ), as well as the very important oxides hematite ( $\text{Fe}_2\text{O}_3$ ) and magnetite ( $\text{Fe}_3\text{O}_4$ ). A series of preliminary experiments with mixtures of iron oxides and sulfur in evacuated, sealed silica tubes have reproduced some of the interesting mineral assemblages common

hematite, and pyrite form a stable assemblage, and hematite may be transformed into pyrite and  $\text{SO}_2$  gas by introduction of sulfur. Further, hematite and  $\text{SO}_2$  may be produced from pyrite by introduction of oxygen, as perhaps takes place on the Isle of Elba. It is seen that hematite below

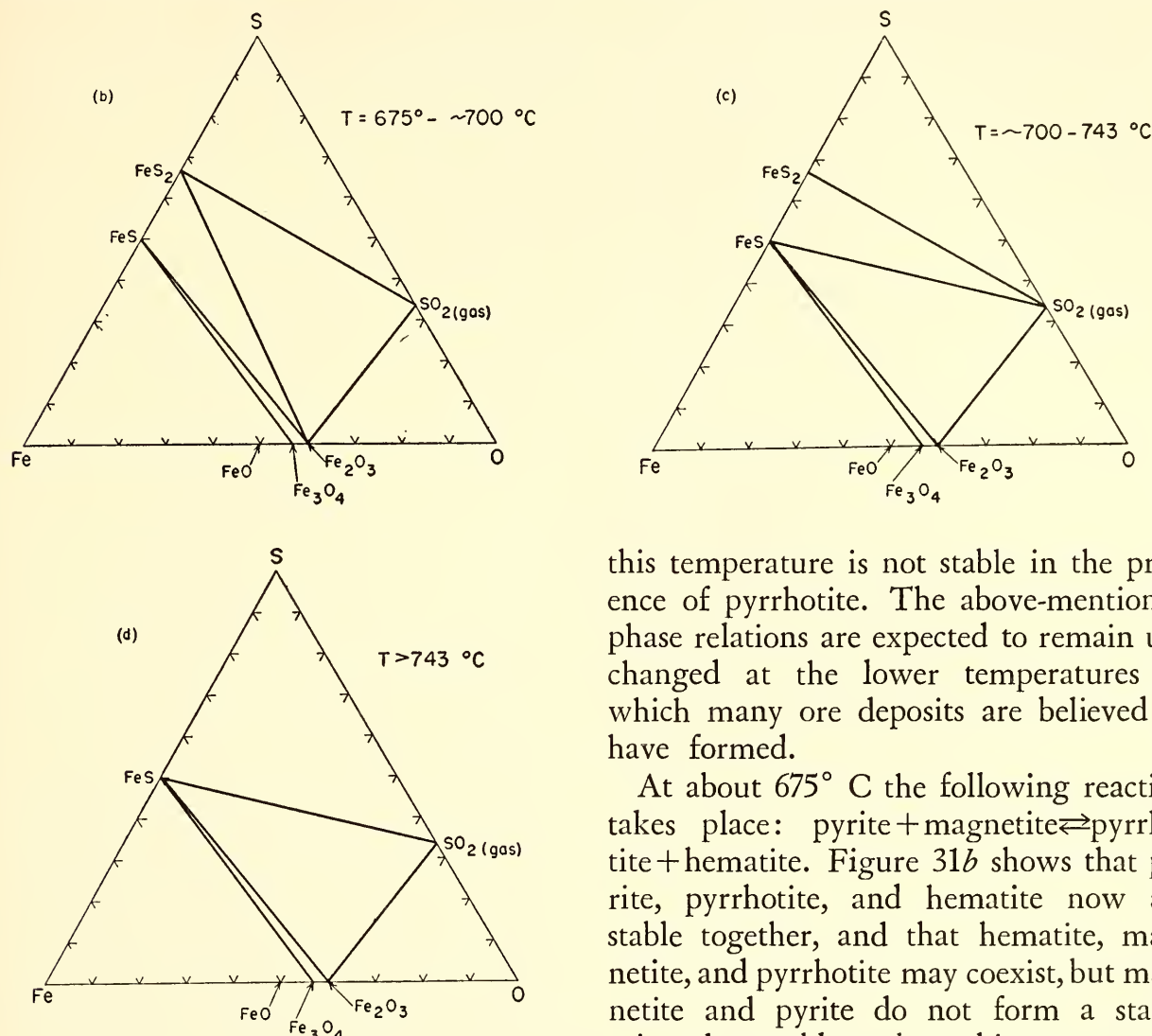


Fig. 31. Phase relations in the Fe-S-O system: (b) between about 675° and 700° C, (c) between 700° and 743° C, (d) above 743° C.

in nature. In the following discussion of the phase relationships shown in figure 31, solid solutions in FeS,  $\text{Fe}_2\text{O}_3$ , and  $\text{Fe}_3\text{O}_4$  have been neglected.

Figure 31a shows that at temperatures below about 675° C pyrite and pyrrhotite can coexist with magnetite. This relationship has been observed in localities such as the Coeur d'Alene district. Magnetite,

this temperature is not stable in the presence of pyrrhotite. The above-mentioned phase relations are expected to remain unchanged at the lower temperatures at which many ore deposits are believed to have formed.

At about 675° C the following reaction takes place: pyrite + magnetite  $\rightleftharpoons$  pyrrhotite + hematite. Figure 31b shows that pyrite, pyrrhotite, and hematite now are stable together, and that hematite, magnetite, and pyrrhotite may coexist, but magnetite and pyrite do not form a stable mineral assemblage above this temperature.

Further changes in the phase relations occur at about 700° C with the reaction: pyrite + hematite  $\rightleftharpoons$  pyrrhotite +  $\text{SO}_2$ . Figure 31c shows that neither hematite nor magnetite now is stable with the pyrite + pyrrhotite assemblage, whereas pyrrhotite, hematite, and magnetite still can coexist.

At 743° C pyrite is no longer stable in a rigid silica tube regardless of the amount of sulfur present. (See discussion of the stability limits of pyrite elsewhere in this report.) Figure 31d shows the phase rela-



tions above 743° C, where pyrite no longer exists. Hematite, magnetite, and pyrrhotite remain a stable mineral assemblage. The geological significance of these phase relations is far-reaching, since these minerals are of almost ubiquitous occurrence.

#### THE Fe-S-Se SYSTEM

*G. Kullerød and H. L. Barnes*

Until recently the selenide minerals had received relatively little attention from geologists and laboratory workers, but the rapidly developing industrial and electronic applications of selenium have focused increasing attention on the modes of occurrence of this element. Thus, ferroselite ( $\text{FeSe}_2$ ), which was first recognized as a mineral about 2 years ago, has already been reported from 15 to 20 localities on the Colorado Plateau alone. By chemical analyses of natural minerals,  $\text{FeSe}_2$  and  $\text{FeS}_2$  (pyrite) have been found to enter into solid solution with each other. These  $\text{Fe}(\text{S,Se})_2$  mix-crystals, which often occur with uranium minerals, may ultimately serve as geological temperature indicators when the  $\text{FeS}_2$ - $\text{FeSe}_2$  system has been studied in the laboratory. The present investigation is also designed to explore the distribution of selenium at various temperatures and pressures between pyrrhotite and pyrite.

Stoichiometric  $\text{FeSe}$  was synthesized at various temperatures from 325° to 925° C. X-ray diffraction patterns made at room temperature showed that eskebornite in this temperature range is of the NiAs structure type (provided that nonquenchable polymorphs do not exist).  $\text{FeSe}$  and  $\text{FeS}$ , therefore, are isostructural.  $\text{FeSe}$ , like  $\text{FeS}$ , can omit iron from its structure, and the chemical formula of natural eskebornite, therefore, should be written  $\text{Fe}_{1-x}\text{Se}$ . Although  $\text{FeS}$ , according to the literature, cannot take excess iron in solid solution, eskebornite was found to take about 2 weight per cent iron in solid solution at 800° C. Mixtures of  $\text{FeS}$  and  $\text{FeSe}$  were

made up at 10 weight per cent intervals and heated at 800° C for 60 days. X-ray diffraction and polished-section studies showed that the solubility of  $\text{FeSe}$  in  $\text{FeS}$  at this temperature is between 30 and 40 weight per cent. The solubility of  $\text{FeS}$  in  $\text{FeSe}$  is between 40 and 50 weight per cent.

X-ray diffraction studies of  $\text{Fe}(\text{S,Se})$  mix-crystals of varying composition show appreciable variation in the (102)  $d$  spac-

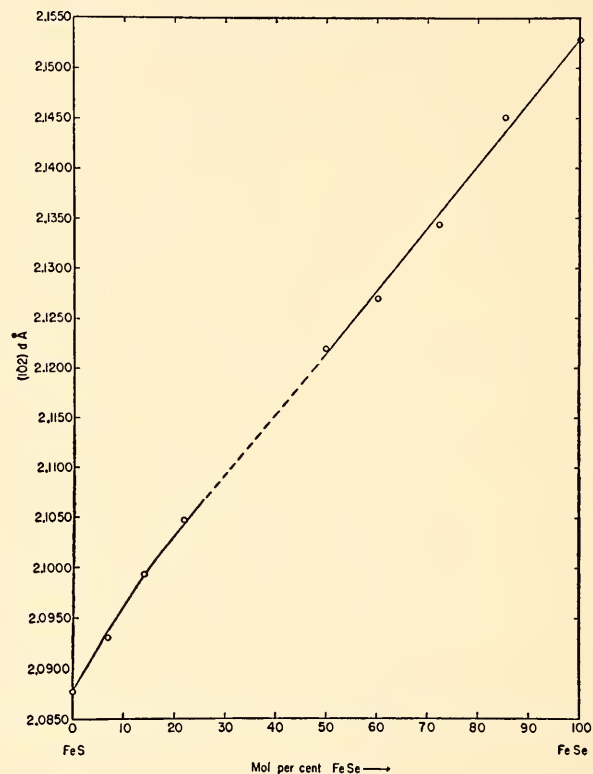


Fig. 32. The curve showing variation in  $d$  spacings for the (102) X-ray reflections as function of mix-crystal  $\text{Fe}(\text{S,Se})$  composition.

ings. The (102)  $d$  spacings versus composition have been plotted in figure 32. Sodium chloride ( $a=5.62869$  kX) was used as internal standard for all measurements. The dotted part of the spacing curve shows where the immiscibility gap occurs at 800° C.

Experiments with  $\text{FeS}$  and  $\text{FeSe}$  mixtures at temperatures between 850° and 400° C established the limits of the binary solid solution within a few per cent. The top of the solvus curve is situated at about 850° C and at a composition of approximately 55 weight per cent  $\text{FeS}$  and 45

weight per cent FeSe. The solubility of FeSe in FeS decreases rapidly with decreasing temperature, and at 600° C is about 10 weight per cent. The solubility of FeS in FeSe at 600° C is about 30 weight per cent. The two-phase field in this system is not binary. Beyond the limit of FeS solubility in FeSe and of FeSe solubility in FeS the two phases  $\text{Fe}_{1-x}(\text{S,Se})$  and  $\text{Fe}_{1+x}(\text{S,Se})$  coexist. The amount of iron deficiency in the first and excess of

The crystal chemistry of these arsenides is of particular interest, as most chemical analyses have suggested that compositions with marked departures from stoichiometric proportions might be common. The different arsenides are commonly zoned or intergrown with one another, however, and can be distinguished only by means of the polarizing reflecting microscope or X-ray techniques. The few analyses that have been made on material checked for

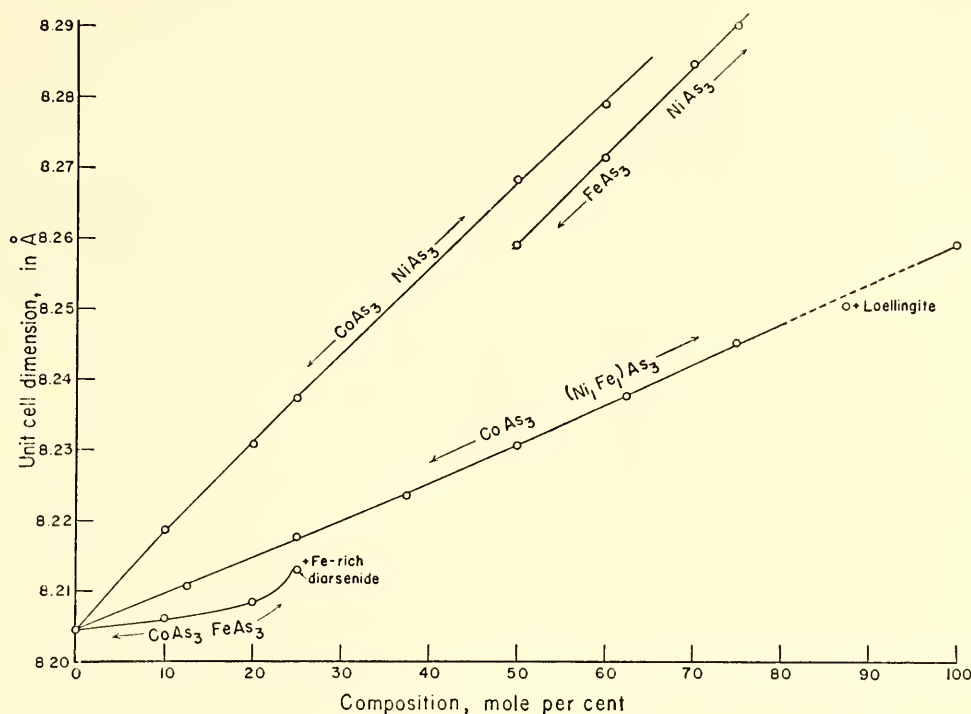


Fig. 33. Curves showing unit cell dimensions of synthetic skutterudites as a function of cobalt-to-nickel ratio, cobalt-to-iron ratio, and cobalt-to-nickel-and-iron ratio (where the nickel-to-iron ratio is 1:1).

iron in the second of these compounds at various S:Se ratios must be carefully studied before the tie lines can be determined in the ternary system.

#### THE $\text{CoAs}_2\text{--NiAs}_2\text{--FeAs}_2\text{--As}$ SYSTEM

*E. H. Roseboom, Jr.*

Ore deposits mined for arsenic, cobalt, nickel, and silver, although not abundant, are of world-wide distribution and constitute a distinctive type. They are remarkably low in sulfur, with cobalt, nickel, and iron present as arsenides or sulfarsenides and with silver and bismuth present in the native state.

homogeneity have indicated nearly stoichiometric compositions for diarsenides, but metal-to-arsenic ratios as low as 1:2.65 for skutterudites,  $(\text{Co,Ni,Fe})\text{As}_{3-x}$ . Such ratios might vary sufficiently with temperature to provide a means of determining the temperature of formation of ore deposits.

Very little was known about the system Co-Ni-Fe-As, which contains eight naturally occurring minerals. The present study was undertaken to explore the portion of the system pertaining to natural deposits. It was hoped that phase changes suitable for establishing limits of temperature and



pressure during formation might be discovered for this group of minerals. As most natural skutterudites contain cobalt, iron, and nickel, and as so little was known of the phase relations, it was thought that a reconnaissance of the arsenic-rich portion of the quaternary system would be of more value than a detailed study of a binary or ternary system in the quaternary. In preliminary attempts to make skutterudites of mixed cobalt, iron, and nickel

amount of arsenic that combined with the metal was determined by weighing the inner tube after the run. The contents of the tube were reground and reheated in the same way until there was no further increase in weight. The maximum arsenic contents given below for cobalt skutterudite ( $\text{CoAs}_{3-x}$ ), loellingite ( $\text{FeAs}_2$ ), and rammelsbergite ( $\text{NiAs}_2$ ) are each the average and standard deviation of three samples. The minimum arsenic contents

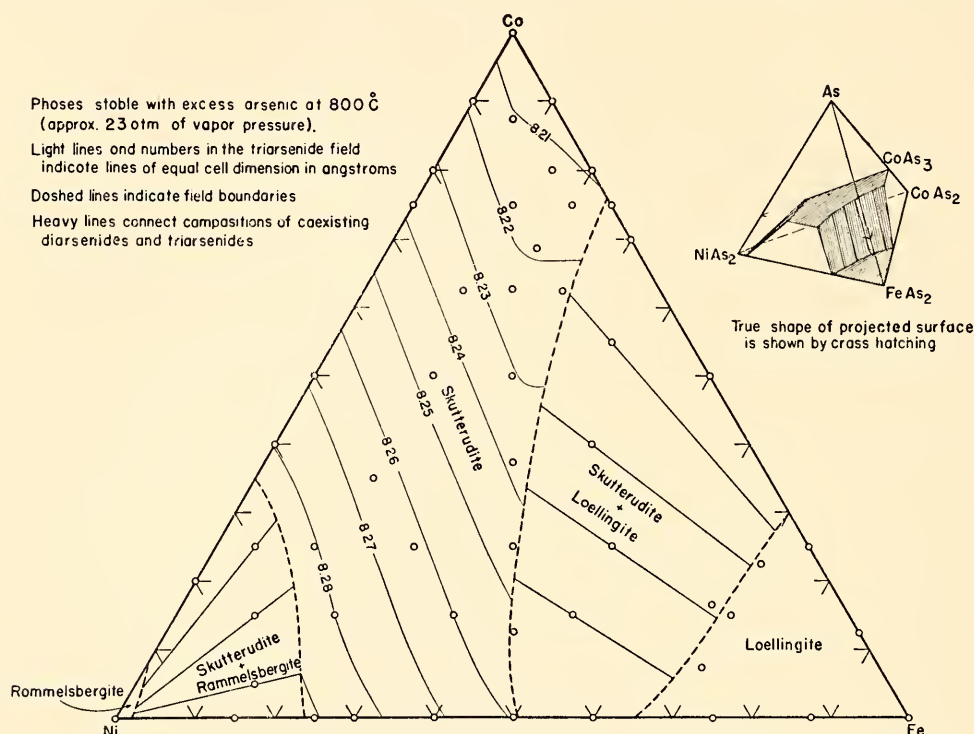


Fig. 34. The cobalt-nickel-iron content of solid phases in the system Co-Ni-Fe-As which are stable with excess arsenic and a vapor phase at 800°C.

composition, it was decided to work at 800°C after runs of several days' duration at 600°, 700°, and 750° C failed to approach equilibrium.

The present work is an isothermal projection of the solid phases stable with a vapor at 800° C and with total pressure equal to the equilibrium vapor pressure of the solid phases present. The runs were made in evacuated sealed silica-glass tubes.

The maximum arsenic content was determined for the phases stable with arsenic by heating at 800° C a known amount of metal in an open tube inside a larger closed tube containing excess arsenic. The

were determined by making runs of successively lower arsenic content until the presence of another phase was detected by microscopic examination of polished sections of the samples. The arsenic contents determined in this manner may be too large by as much as 0.01, as a much larger proportion of arsenic than cobalt is involved in the vapor phase.

At 800° C cobalt skutterudite has a minimum arsenic content between  $\text{CoAs}_{2.94}$  and  $\text{CoAs}_{2.95}$  and a maximum of  $\text{CoAs}_{2.960 \pm 0.001}$ . Loellingite has a lower limit between  $\text{FeAs}_{1.97}$  and  $\text{FeAs}_{1.98}$  and an upper limit of  $\text{FeAs}_{1.998 \pm 0.002}$ . Rammels-





bergite has a lower limit above  $\text{NiAs}_{1.99}$  and an upper limit of  $\text{NiAs}_{1.999 \pm 0.001}$ . In no case was there a measurable difference in X-ray  $d$  spacings between the phases made with excess arsenic and the same phases made with deficient arsenic.

The name skutterudite is used for all cubic "triarsenides" of cobalt, iron, and nickel. Thus the formula may be expressed approximately as  $(\text{Co,Fe,Ni})\text{As}_{3-x}$ . Skutterudites of varied cobalt, iron, and nickel content were made in the presence of excess arsenic. Figure 33 shows the effect of composition on cell size for various series of skutterudites. Figure 34 shows the cobalt-iron-nickel content of phases stable with excess arsenic at  $800^\circ\text{C}$ . The lines of equal cell dimension for the skutterudites are also shown. In the iron and nickel corners of the triangle there are fields in which loellingites and rammelsbergites of varied compositions are stable with excess arsenic. Between these fields and the skutterudite field, a skutterudite is stable with either a loellingite or a rammelsbergite, plus arsenic.

Runs made at  $800^\circ\text{C}$  in the diarsenide + skutterudite + arsenic fields and annealed at  $600^\circ$  and  $700^\circ\text{C}$  for 3 months indicated that the limits of the binary series of skutterudites decrease by 1 to 3 per cent except for the iron-rich end of the nickel-iron skutterudites, which increases to 55 per cent iron 45 per cent nickel at  $600^\circ\text{C}$ .

The limits of iron, cobalt, and nickel content of these artificial skutterudites contain 87 per cent of the remaining analyses for natural skutterudites compiled from the literature by Holmes (1947) after he had eliminated analyses made on material that was probably not skutterudite. Only 6 per cent of these analyses were more than 5 per cent beyond the limits.

Although both loellingite and rammelsbergite are orthorhombic, cobalt diarsenide appears to be monoclinic. The evidence for this was the splitting of the 110 and 111  $d$  spacings into two spacings. The split begins at about 25 per cent  $\text{NiAs}_2$  75 per cent  $\text{CoAs}_2$  and at about 20 per cent

$\text{FeAs}_2$  80 per cent  $\text{CoAs}_2$ , and reaches a maximum at  $\text{CoAs}_2$ .  $\text{NiAs}_2$  and  $\text{CoAs}_2$ , and  $\text{FeAs}_2$  and  $\text{CoAs}_2$ , apparently form a complete solid solution series. The series  $\text{NiAs}_2$ - $\text{FeAs}_2$  is discontinuous at  $800^\circ\text{C}$  because of an asymmetrical solvus with a crest estimated at about  $900^\circ\text{C}$  and about 15 per cent  $\text{FeAs}_2$  85 per cent  $\text{NiAs}_2$ .

The condensed diagram of the systems  $\text{CoAs}_2$ - $\text{NiAs}_2$ -As,  $\text{NiAs}_2$ - $\text{FeAs}_2$ -As, and  $\text{FeAs}_2$ - $\text{CoAs}_2$ -As at  $800^\circ\text{C}$  is shown in figure 35 as the unfolded sides of a tetrahedron with arsenic at the apex. The diagram is based on relatively few runs. After the diarsenide and skutterudite solid solution series were established, it was possible to locate the corners of the three-phase fields from the  $d$  spacings of the phases. As arsenic melts at about  $818^\circ\text{C}$  under its own vapor pressure, a eutectic between arsenic and the arsenides of highest arsenic content might extend below  $800^\circ\text{C}$ . Hence it is possible that at  $800^\circ\text{C}$  there are some liquid fields in the arsenic-rich portion of the diagram, although no evidence of them was observed.

#### RELATIONS BETWEEN COMPOSITION OF ORE MINERALS AND ORE SOLUTIONS

*H. L. Barnes and G. Kullerud*

Economic geologists and laboratory scientists agree that the majority of the world's sulfide ore bodies must have been deposited from aqueous solutions or fluids. These aqueous fluids must have contained metals and sulfur combined in the form of complex ions. Such complexes could break down as the result of changes in physical and chemical conditions, leading to sulfide deposition.

In some ore bodies limits can be placed on the conditions existing at the time of ore formation on the basis of subsolidus relations and mineral solubilities, but even where the phase relations give relatively accurate physical data, the chemistry involved during transport and precipitation is not well understood. A complete understanding of the chemistry of such trans-

porting agents could undoubtedly be developed into a powerful tool for prediction of ore occurrence.

Although the considerable economic and theoretical importance of an understanding of the chemistry of ore solutions has been clear to numerous workers in the field of geology, the ore solutions involve extremely complicated chemistry which of necessity has been commonly oversimplified in its treatment.

Thus, many recent papers using mineral compositions for data on the composition of hydrothermal ore solutions have been based on simple ionic chemistry without a clear statement of the implicit assumptions involved. By summarizing the physical and chemical conditions which must necessarily be evaluated, these assumptions become self-evident:

1. Equilibrium conditions must occur during deposition of the minerals. In other words, metastable phenomena such as supersaturation or coprecipitation must not modify conditions of precipitation, or calculations of equilibria would be meaningless.

2. If the ratio of concentrations of any given element in two minerals is to be used for defining the ore solution, the two minerals must have been precipitated under physically and chemically identical conditions. Practically, this limitation requires either that the two minerals were precipitated simultaneously or that there has been no change in the composition, pressure, or temperature of the ore solution between the time of precipitation of the first and second mineral.

3. There must be no post-depositional changes in the composition of mix-crystals used for data on depositional environment, though these mineral compositions may be far out of equilibrium with subsequent environments. Neither exsolution nor solution is permissible, although both processes are favored by recrystallization or changes in chemical environment, pressure, or temperature, especially during slow

cooling of deep-seated deposits or heating in later metamorphism.

4. The relative distribution and types of ions present in the ore solution must be known. Specifically, information on the extent to which dissociation, hydrolysis, and complexing take place is important in order to estimate the total ionic strength and to ascertain the metal transporting ions.

5. Activity coefficients of both the aqueous ions and the mix-crystals need to be evaluated as functions of the four independent variables temperature, pressure, ionic species, and ionic strength.

Lack of information necessitated the assumption of specific conditions for theoretical treatment even without direct evidence. Geologic data on the history of a particular mineral sample are very difficult to collect, and, at present, there exist virtually no chemical data obtained under the difficult experimental conditions approximating those at which ore deposits were formed.

Knox (1908) and Dickson and Tunell (1955) have studied the chemical behavior of  $\text{HgS}$  under varied chemical conditions simulating ore transport. Experimental work on the solubility of  $\text{ZnS}$  in  $\text{H}_2\text{O}$  under elevated pressures and temperatures in a  $\text{H}_2\text{S}$  atmosphere is now in progress in this Laboratory.

If sufficient data were available, four simultaneous equations could be derived to relate these four independent variables to mix-crystal concentration ratios. For a unique solution at least four accurate concentration ratios are necessary. These ratios may be of two types: (*a*) the fractional content of one trace element in one mineral may be expressed and used as a ratio provided that there is an excess of the trace element present at the time of deposition to assure that the mineral is saturated with the trace element at that temperature and pressure; and (*b*) the distribution ratio of one trace element between two minerals may be used where neither is saturated with the trace element. The four



ratios required could be measured theoretically for any combination of the two types where the distribution ratio could be measured for one trace element in several minerals, several trace elements in the same two minerals, or any intermediate combination.

From a practical point of view, the trace-element concentration in a mineral can be used only if it is sufficiently high for accurate analysis and if the mineral can be

effectively separated from potentially contaminating foreign minerals. In order to use these ratios, the minerals involved must have been deposited contemporaneously. It is doubtful whether minerals fulfilling these conditions and providing a sufficient number of ratios for the solution of the simultaneous equations can be found in actual ore deposits; it remains a crucial question, however, and must be investigated.

## FELDSPARS

### TERNARY FELDSPARS

*H. S. Yoder, D. B. Stewart, and J. R. Smith*

The ternary feldspar study briefly outlined last year has advanced to a very fruitful stage. The relations of the various feldspars, of greatest import to the petrologist, may now be defined more closely and the results applied to natural rocks. The principal feldspars lie in the system  $\text{NaAlSi}_3\text{O}_8$  (Ab)– $\text{KAlSi}_3\text{O}_8$  (Or)– $\text{CaAl}_2\text{Si}_2\text{O}_8$  (An); this system has been investigated with water under a pressure of 5000 bars, mainly using glasses prepared by Franco, Schairer, and Bowen.

*Projections* of the bounding ternary systems Ab–An– $\text{H}_2\text{O}$ , Or–An– $\text{H}_2\text{O}$ , and Ab–Or– $\text{H}_2\text{O}$  are given in figures 36, 37, and 38, respectively. The uncertainty of the nature of the melting of compositions near anorthite mentioned last year has been resolved. The “ $\beta$ -alumina” of unknown composition that appeared in some runs has dissolved with longer runs, and it may be stated with confidence that anorthite melts congruently at 5000 bars water pressure. Much effort was put into fixing the limits of the solvus in the Or–Ab– $\text{H}_2\text{O}$  system (fig. 38). The solvus as pictured is based on 1-month runs using only the results from glass starting materials. The number of phases was identified by means of powder X-ray diffraction patterns, since optical recognition was not always possible. Equilibrium was demonstrated by holding previously crystallized glasses known to consist of a single phase at the same tem-

peratures and pressure. In the unmixing region as outlined by runs using glass as a starting material most of the compositions began to unmix. Unfortunately, single feldspars formed previously from a glass did not unmix to the same compositions of feldspars as those obtained from glass crystallized in the unmixing region even in a month's time on the basis of the  $20\bar{1}$  spacings of the X-ray diffraction pattern. The  $20\bar{1}$  spacings do not give a suitable measure of the composition in unmixed feldspars, according to Coombs and others, and so the  $20\bar{1}$  spacing could be used as only an approximate measure of the attainment of equilibrium. However, the incompleteness of unmixing of those single feldspars previously formed from glass was well outside the errors of estimating the composition by the  $20\bar{1}$  spacing. From runs made to test the possibility of a solvus in the Ab–An– $\text{H}_2\text{O}$  system, only single-phase crystalline products were obtained. The peristerites may be the result of complex phase relationships arising in the Ab–Or–An system.

Because the lines in the diagrams in figures 36, 37, and 38 are *projections* of boundary surfaces between assemblages in equilibrium with gas, they cannot be read in the same manner as anhydrous diagrams of similar appearance. In order to illustrate this fact, two *sections* determined experimentally at constant temperature and pressure are given for Ab–Or– $\text{H}_2\text{O}$ , one of the bounding systems. In figure 39 is

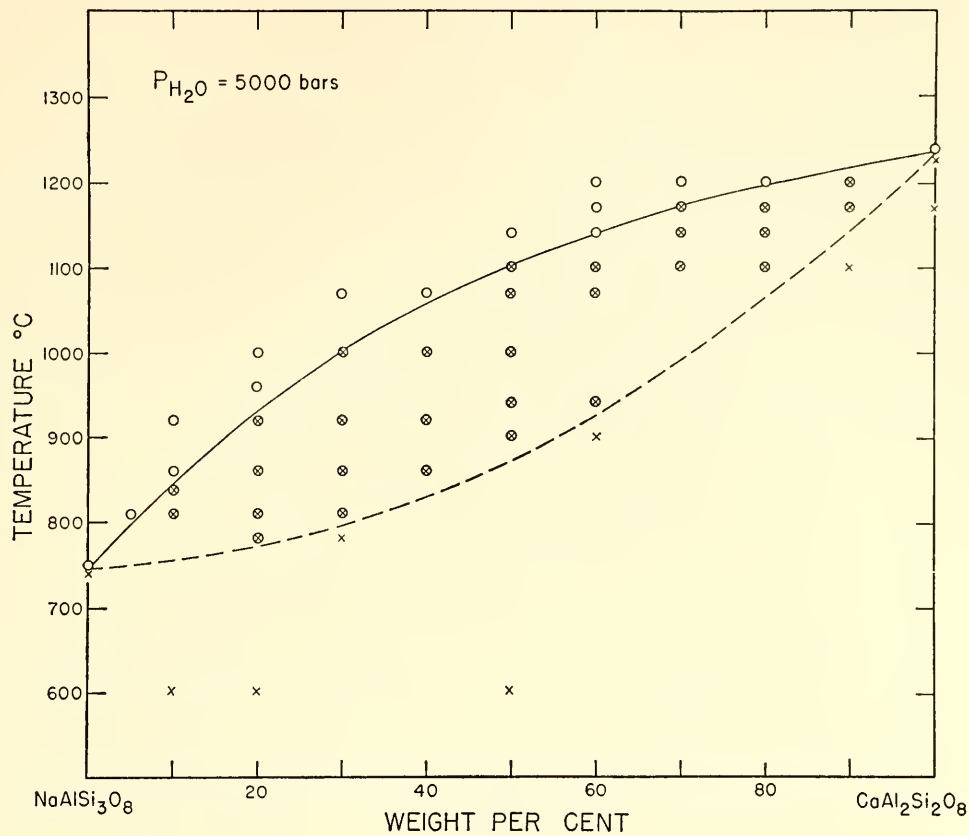


Fig. 36. Projection of the ternary system  $\text{NaAlSi}_3\text{O}_8$ - $\text{CaAl}_2\text{Si}_2\text{O}_8$ - $\text{H}_2\text{O}$  at 5000 bars  $\text{H}_2\text{O}$  pressure.

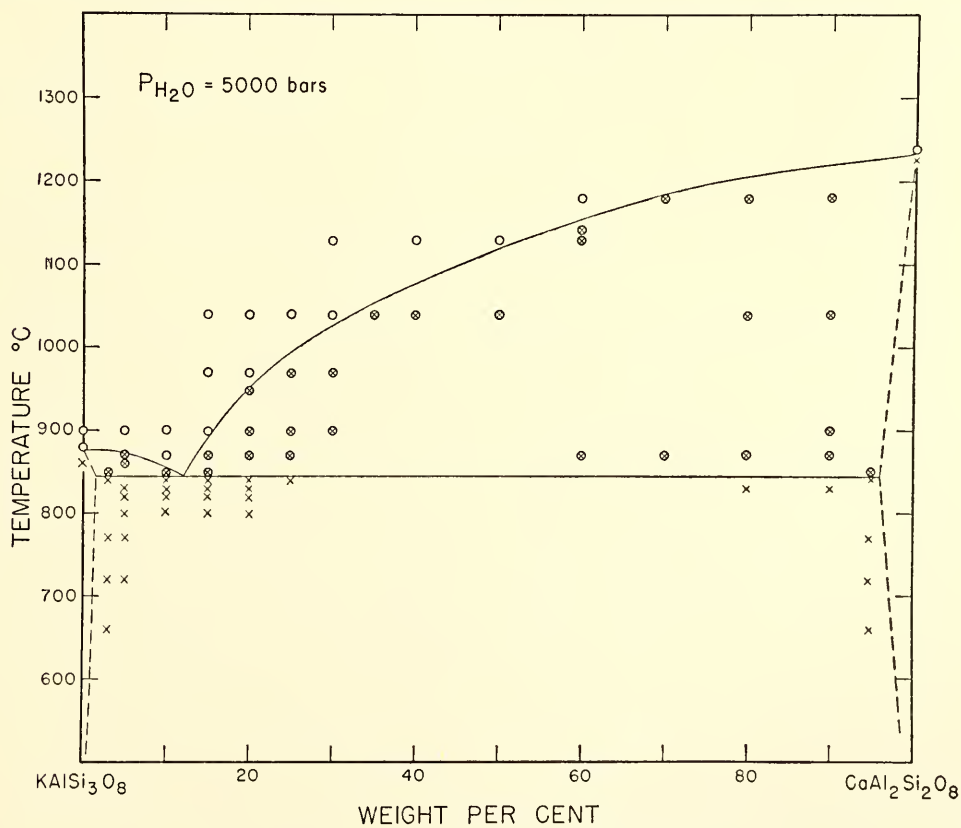


Fig. 37. Projection of the ternary system  $\text{KAlSi}_3\text{O}_8$ - $\text{CaAl}_2\text{Si}_2\text{O}_8$ - $\text{H}_2\text{O}$  at 5000 bars  $\text{H}_2\text{O}$  pressure.



the 720° C, 5000 bar section, and all these data project onto the 720° C line in figure 38. Attention is called to the large amount of H<sub>2</sub>O in the liquids (~11 per cent) and the resulting large area in which a gas phase is prohibited. The second *section*, figure 40, at 710° C, 5000 bars, shows somewhat the same relations, and in addition indicates that the solvus has been transected in the region where a gas phase is prohibited. The maximum on the solvus does not appear on the projection (fig. 38), since

that they contain about 1 to 2 per cent of the feldspar components (see figs. 39 and 40).

With the knowledge gained from the three bounding ternary systems, it was then possible to study the quaternary Ab-Or-An-H<sub>2</sub>O at 5000 bars. If the problem is described loosely in terms of the anhydrous systems, the study consisted of tracing the way in which the three fundamental bounding systems, one of the continuous-series type (fig. 36), another of the eutec-

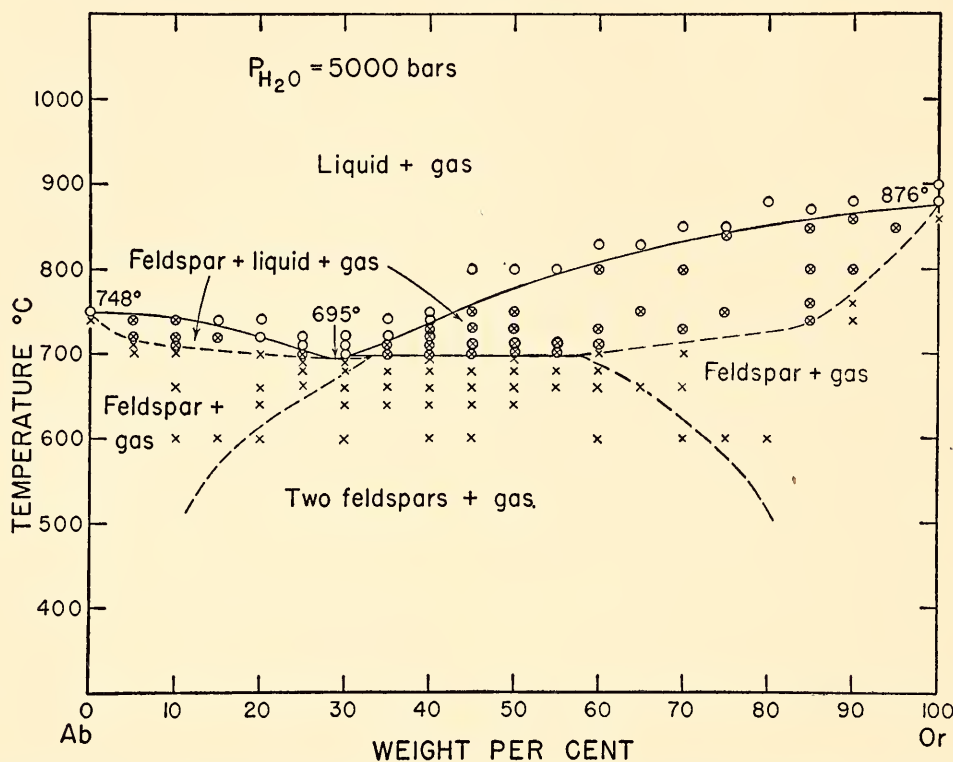


Fig. 38. Projection of the ternary system NaAlSi<sub>3</sub>O<sub>8</sub>-KAlSi<sub>3</sub>O<sub>8</sub>-H<sub>2</sub>O at 5000 bars H<sub>2</sub>O pressure.

only those assemblages in equilibrium with gas are indicated. On the basis of the data in these two sections, the maximum on the solvus at 5000 bars *total* pressure is placed at 715° ± 5° C and Ab<sub>55</sub>Or<sub>45</sub> ± 3 weight per cent. Bowen and Tuttle determined the maximum of the solvus at 1000 kg/cm<sup>2</sup> to be 660° C and Ab<sub>55</sub>Or<sub>45</sub>, using the 20 $\bar{1}$  method for two points and determining the number of phases for a third point at the maximum. With the latter datum, the pressure raises the maximum on the solvus about 14°/1000 bars. Although the composition of the gases has not been studied in great detail, preliminary results suggest

tic type (fig. 37), and the third of the minimum type (fig. 38), merge in *T-X* space. The projection of the determined liquidus diagram when gas is present is given in figure 41. One of the most significant features is the generally low temperatures, temperatures readily available in the crust, as compared with the anhydrous system determined by Franco and Schairer. Also of importance is the OrAb-rich nature of the liquids along the four-phase boundary curve. This observation has bearing on many geological problems, particularly those involving partial melting or metasomatism. The four-phase bound-

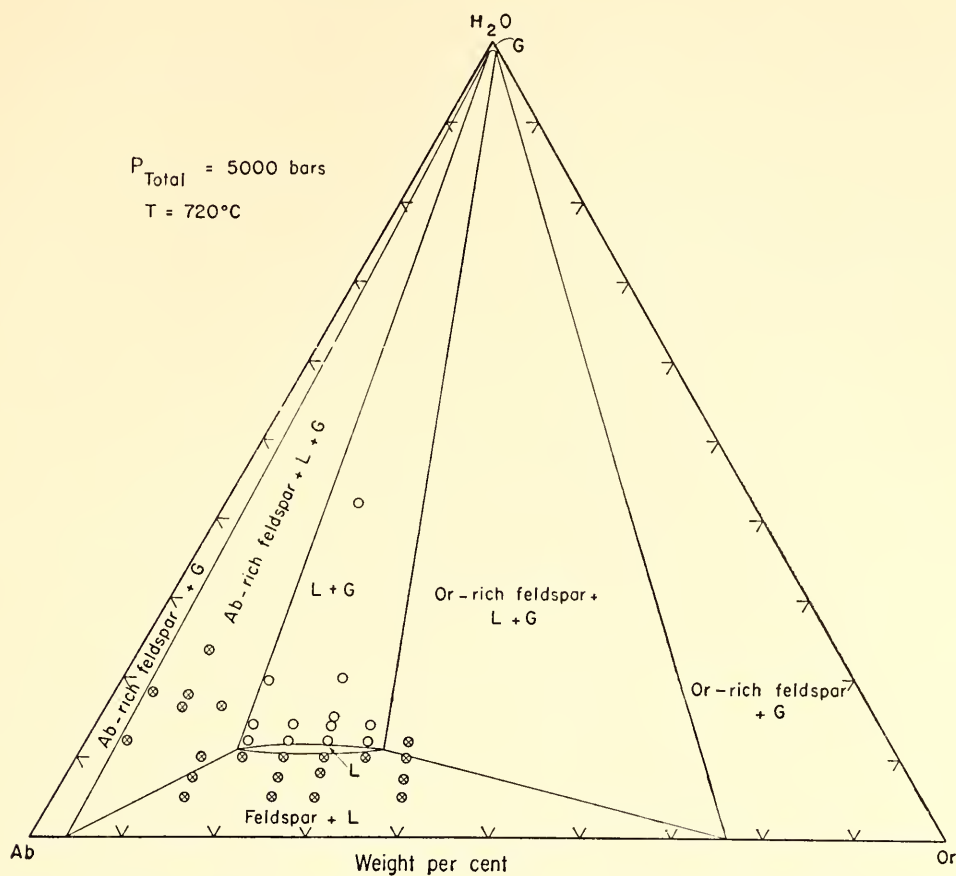


Fig. 39. The  $720^\circ\text{C}$  section at 5000 bars of the system  $\text{NaAlSi}_3\text{O}_8$  (Ab) -  $\text{KAlSi}_3\text{O}_8$  (Or) -  $\text{H}_2\text{O}$ .

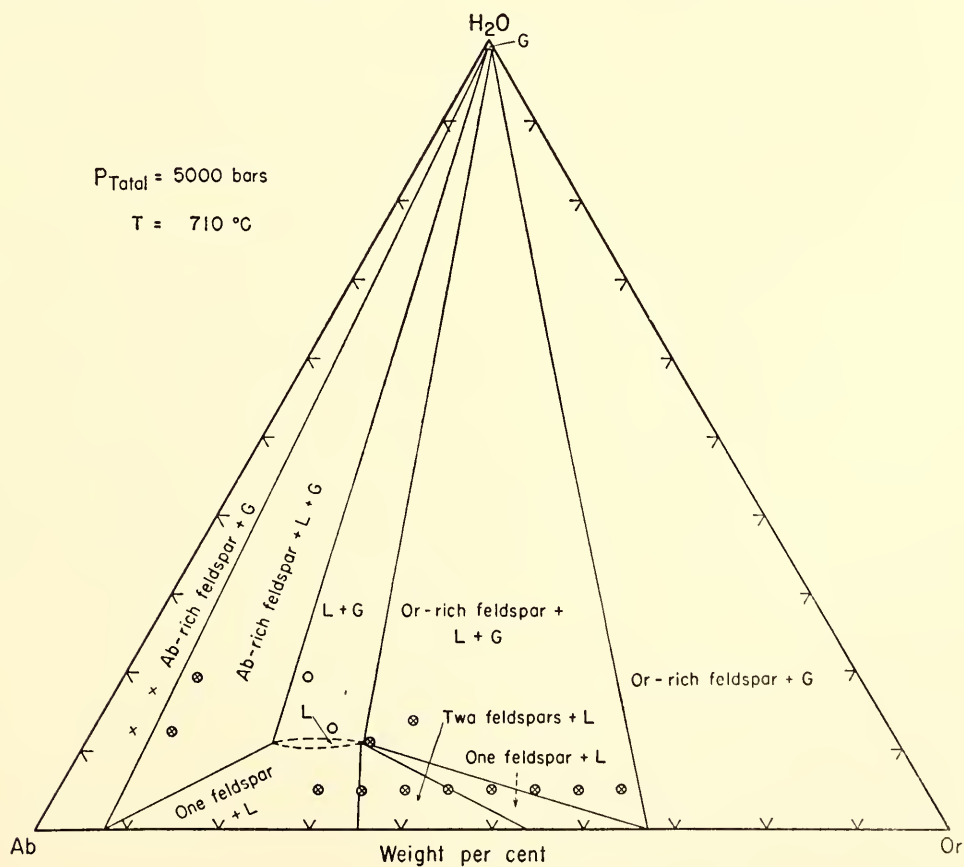


Fig. 40. The  $710^\circ\text{C}$  section at 5000 bars of the system  $\text{NaAlSi}_3\text{O}_8$  (Ab) -  $\text{KAlSi}_3\text{O}_8$  (Or) -  $\text{H}_2\text{O}$ .



ary curve becomes a three-phase trough (indicated by short dash) at a fixed temperature between  $695^{\circ}$  and  $698^{\circ}$  C. The change takes place at the temperature at which two feldspars react with liquid and gas to produce one feldspar, liquid, and gas. The one feldspar produced has the composition of the point of contact of the solidus and the solvus.

In order to understand more fully the relations in the complex quaternary system, three isothermal, isobaric projections have been studied. The  $770^{\circ}$  C, 5000 bar projection, given in figure 42, presents only those assemblages that are in equilibrium with gas. The chief observations are the composition of feldspars in equilibrium with liquid and gas and the orientation of the tie line (light line) which separates the two feldspars + gas field from the two feldspars + liquid + gas field. The tie lines that connect coexisting feldspars or coexisting feldspar and liquid in the three-phase regions do not appear on the diagram, since no data are known for fixing the composition of ternary feldspars except along univariant curves or at invariant points. At invariant points or along univariant curves the points were fixed by observing the number and kind of phases about the point.

In figure 43 the relations of the  $720^{\circ}$  C, 5000 bar projection are given for only those phases in equilibrium with gas. The orientation of the tie line fixing the composition of the two feldspars in equilibrium with liquid and gas should be noted and compared with the similar tie line in figure 42. In general, the change of composition of the AbAn-rich feldspar with temperature is greater than that change for the OrAb-rich feldspar. The small triangular area of the projected four-phase region in both isothermal, isobaric projections (figs. 42 and 43) indicates the limited range of temperature through which two feldspars may crystallize simultaneously from a given magma under equilibrium conditions. The range is zero at the Or-An-H<sub>2</sub>O join and near the Or-Ab-H<sub>2</sub>O

join. With fractionation, the range of simultaneous crystallization would be extended.

Some notion as to the way in which the tie lines connecting coexisting feldspars in equilibrium with liquid and gas sweep across the 5000-bar projection may be gained from figure 44. The family of such tie lines, which include the four determined experimentally, generates a surface, the boundary curves (dashed) marking the maximum solid solution of coexisting feldspars in equilibrium with liquid and gas. A third isothermal, isobaric projection is now being investigated at  $660^{\circ}$  C, 5000 bars, to determine the relations when only crystals and gas are present. It is realized that many of the phases obtained in these studies undergo transitions of the first order and of higher orders. Although powder X-ray diffraction patterns have been taken of many of the synthetic crystals, none of the crystals was suitable for single-crystal X-ray study. Minor changes may, therefore, be required in the equilibrium diagrams when sufficient knowledge of the exact structural form of each phase is obtained. In addition, hydrous phases will appear at temperatures lower than those investigated.

In advance of these data the subsolidus regions of one and two feldspars, those regions of most importance to the geologist, may be illustrated schematically. Figure 45 outlines the field boundary surfaces at 5000 bars which separate the two-feldspar region (inside the truncated "dome") from the one-feldspar regions. The top surface of the "dome," the intersection of the solvus and the solidus, represents the experimentally determined relations already given in figure 44. For comparison, a similar picture (fig. 46) is presented for a water pressure of about 2000 bars. This schematic figure was deduced mainly from the work of Bowen and Tuttle on the Ab-Or-H<sub>2</sub>O system at 2000 bars. In figure 46 it is seen that the surface marking the solvus-solidus intersection does not meet the Ab-Or join. The resulting ori-

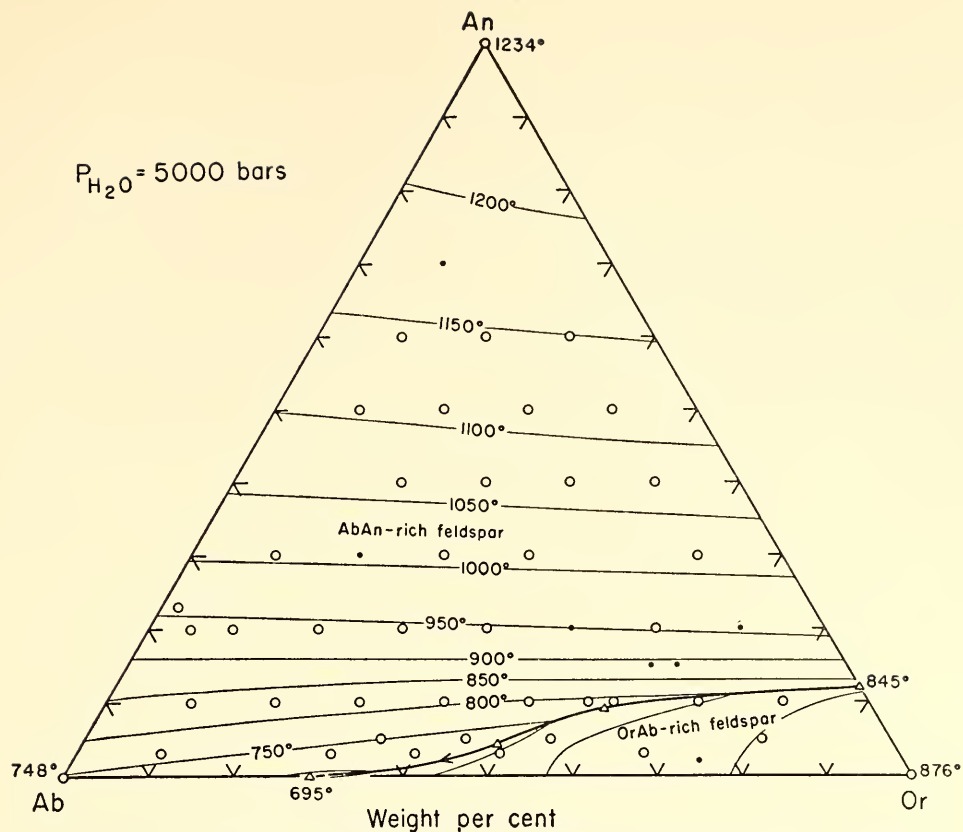


Fig. 41. Projection of the quaternary system  $\text{NaAlSi}_3\text{O}_8$  (Ab) -  $\text{KAlSi}_3\text{O}_8$  (Or) -  $\text{CaAl}_2\text{Si}_2\text{O}_8$  (An) -  $\text{H}_2\text{O}$  at 5000 bars  $\text{H}_2\text{O}$  pressure. The circles indicate compositions for which a bracket was obtained. The dots indicate compositions for which a bracket was not obtained; significant temperatures were investigated, however.

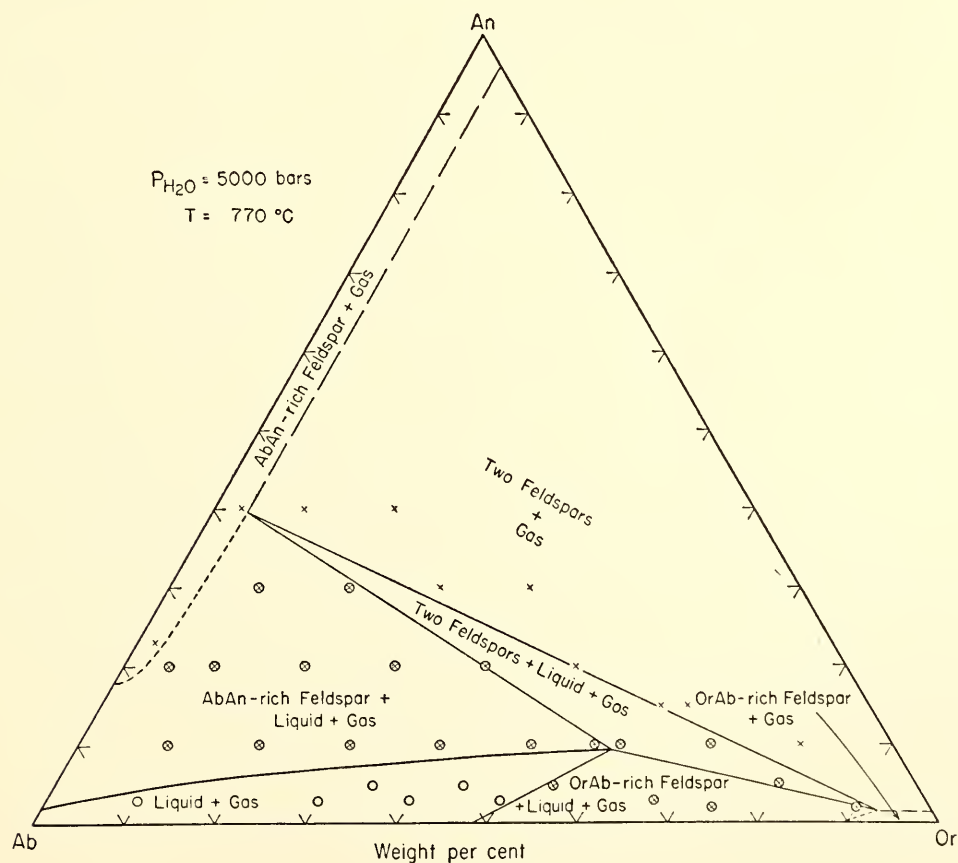


Fig. 42. Projection of the  $\text{NaAlSi}_3\text{O}_8$  (Ab) -  $\text{KAlSi}_3\text{O}_8$  (Or) -  $\text{CaAl}_2\text{Si}_2\text{O}_8$  (An) -  $\text{H}_2\text{O}$  system at 770° C and 5000 bars  $\text{H}_2\text{O}$  pressure. Only those assemblages in equilibrium with gas are given.



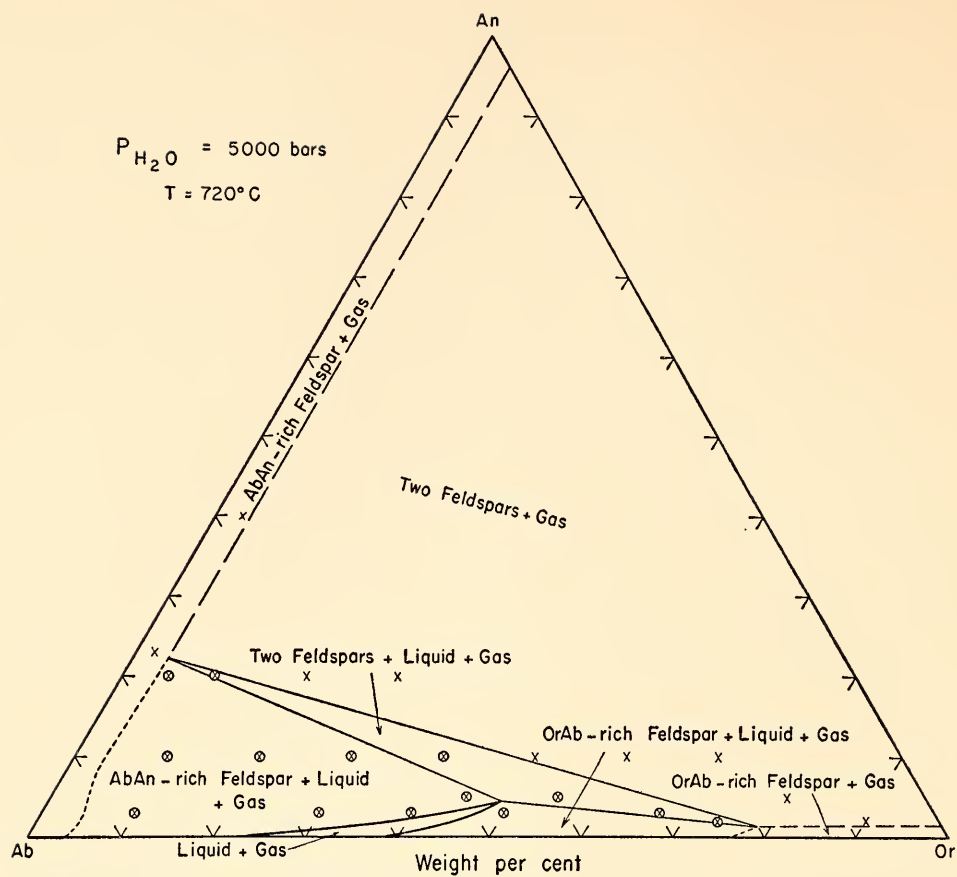


Fig. 43. Projection of the  $\text{NaAlSi}_3\text{O}_8$  (Ab)– $\text{KAlSi}_3\text{O}_8$  (Or)– $\text{CaAl}_2\text{Si}_2\text{O}_8$  (An)– $\text{H}_2\text{O}$  system at  $720^\circ\text{C}$  and  $5000\text{ bars H}_2\text{O}$  pressure. Only those assemblages in equilibrium with gas are given.

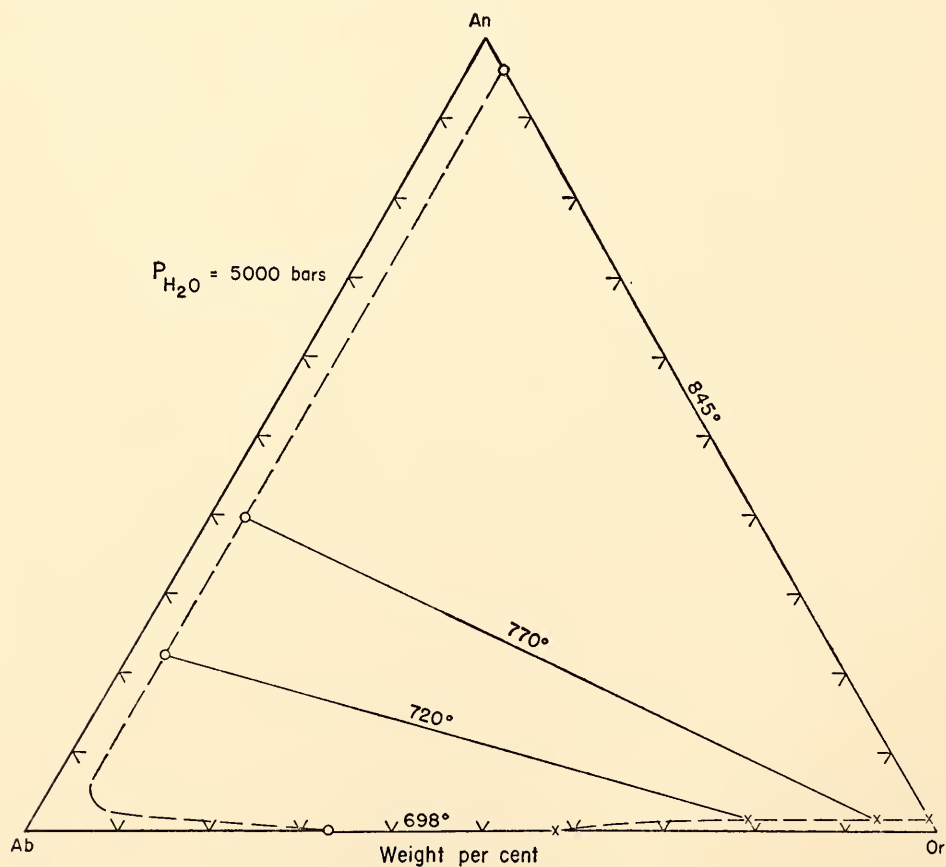


Fig. 44. Plot of experimentally determined tie lines connecting coexisting feldspars in equilibrium with liquid and gas. These appear from the highest to the lowest temperature in figures 37, 42, 43, 38, respectively.

entation of the tie lines connecting coexisting feldspars is therefore different from those at 5000 bars water pressure. Furthermore, the extent of solid solution increases on this surface with lower pressure for a given bulk composition. In general, the tie lines make a smaller angle to the Or-An side at high temperatures and low pressures than at low temperatures and high pressures. If the pressure or temperature were known, then the temperature or pressure of formation, respectively,

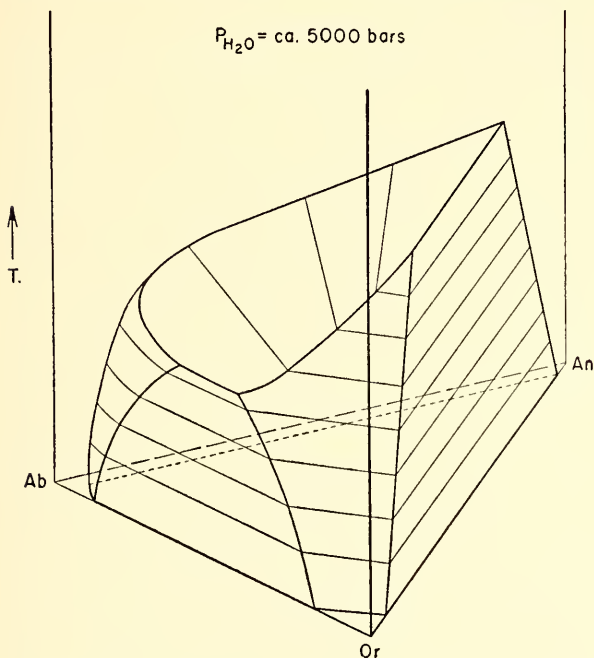


Fig. 45. Schematic presentation of the field boundary surfaces separating the two-feldspar region (inside the truncated "dome") from the one-feldspar and the two-feldspar + liquid + gas regions of the Ab-Or-An-H<sub>2</sub>O system at 5000 bars H<sub>2</sub>O pressure.

could be estimated from knowledge of the compositions of the coexisting feldspars. Since the field geologist can often make an estimate of the depth at which a rock may have formed, assuming that  $P_{H_2O} = P_{total}$ , the compositions of the coexisting feldspars can yield a measure of the temperature of formation, provided that equilibrium was attained. If the pressure of formation cannot be specified, but is believed to be constant, the relative change of the compositions of coexisting feldspars in a series of rocks indicates the relative temperatures of formation.

In figure 47 are assembled some of the analyzed pairs of coexisting feldspars recorded in the literature. Most of the sam-

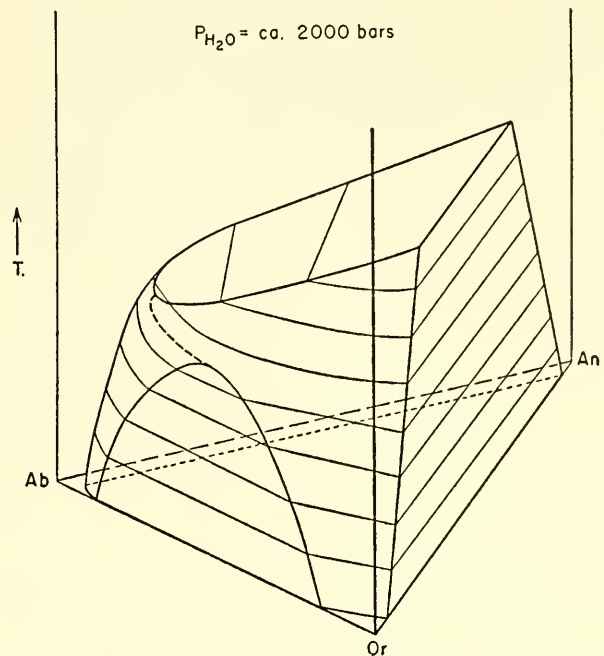


Fig. 46. Schematic presentation of the field boundary surfaces separating the two-feldspar region (inside the "dome") from the one-feldspar and the two-feldspar + liquid + gas regions of the Ab-Or-An-H<sub>2</sub>O system at 2000 bars H<sub>2</sub>O pressure. Based on the work of Bowen and Tuttle (1950) on the Or-Ab-H<sub>2</sub>O system.

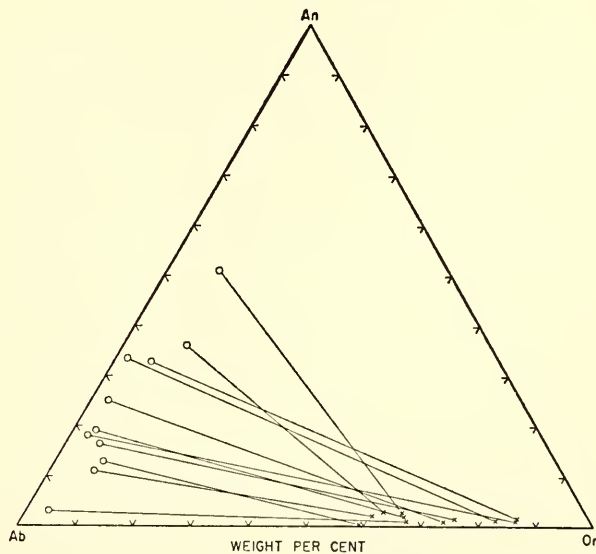


Fig. 47. Plot of chemically analyzed pairs of coexisting feldspars recorded in the literature. Homogeneity of phase has not been tested by X rays in most cases.

ples were not available to test for homogeneity by X rays. The orientations of the tie lines, with the exception of two having the most An-rich plagioclases, are



in accord with those obtained experimentally. The two exceptions are indicative of a lower pressure and a higher temperature; in fact, the feldspars from these specimens are phenocrysts from surface flows. However, another specimen from a surface flow, having a plagioclase of  $An_{11}$ , cannot be readily identified as having formed at low pressure, and it is likely that the properties of these feldspars are inherited from an environment at depth.

The present study, giving quantitative information on the number of feldspars, their relative proportions, and the extent of their solid solutions, emphasizes the need for determining the composition of each feldspar phase in a rock. The compositions of the feldspars, or any pair of minerals sharing components, may yield specific clues to the temperature and pressure of formation. Inasmuch as more than half of the earth's crust is composed of feldspar, the feldspars are potentially most valuable geothermometers and geopiezometers.

#### THE SYSTEM $CaAl_2Si_2O_8-SiO_2-H_2O$

*D. B. Stewart*

Despite the dominance of the feldspars as constituents of the earth's crust, rocks composed entirely of feldspar are not common. In addition to feldspathic minerals, most feldspar-bearing rocks contain significant amounts of either free silica or more rarely a feldspathoid. The phase relations of feldspars and silica have been studied intensively at this Laboratory by Schairer and Bowen, and Tuttle and Bowen have investigated the systems  $NaAlSi_3O_8-SiO_2-H_2O$ ,  $KAlSi_3O_8-SiO_2-H_2O$ , and  $NaAlSi_3O_8-KAlSi_3O_8-SiO_2-H_2O$  ("synthetic granite") to pressures of 4000 bars.

The present study complements these investigations, as the great bulk of all granitic rocks contain the  $CaAl_2Si_2O_8$  component, and the work on ternary feldspars reported in this Year Book indicates the profound effect of small amounts of this component on the phase relations of the feldspars. A feldspar, silica polymorph,

gas, and liquid saturated with  $H_2O$  coexist at a given pressure at only one temperature. One of the purposes of this investigation of the system  $CaAl_2Si_2O_8-SiO_2-H_2O$  is to determine the locus of four-phase points as the pressure is changed. The system  $CaAl_2Si_2O_8-SiO_2-H_2O$ , together with the results of Tuttle and Bowen for the same pressure, fixes the end points of the  $H_2O$ -saturated liquidus where feldspar and silica coexist. At this fixed pressure the assemblage including anorthite as one of the four phases coexists at a higher temperature than any similar assemblage of feldspar, silica, saturated liquid, and gas. To the extent that the three limiting ternary systems are known at various pressures, the direction and magnitude of the changes of the liquidus caused by variations of  $H_2O$  pressure during crystallization can be described.

All the present results were obtained with Yoder's apparatus, and his unpublished results for the system  $CaAl_2Si_2O_8-H_2O$  have been utilized and confirmed. The  $SiO_2-H_2O$  liquidus passes through the points  $1130^\circ \pm 5^\circ$  C at 2000 bars  $H_2O$  pressure and  $1065^\circ \pm 5^\circ$  C at 5000 bars. These data modify and extend the diagram given by Tuttle and England (1955), and such high temperatures and pressures indicate that quartz veins or the quartz cores of pegmatites could not have crystallized *in situ* from a hydrous  $SiO_2$  melt. The rate of lowering of the saturated liquidus in the interval 2000 to 5000 bars is about  $22^\circ$  C per 1000 bars  $H_2O$  pressure, a rate two-thirds of the rate of lowering of the saturated liquidus of any feldspar in the same interval.

A projection of the results at 2000 bars  $H_2O$  pressure is given in figure 48. The only silica polymorph found was high quartz; the saturated liquids contained approximately 6 per cent  $H_2O$  by weight. The four-phase point is at  $922^\circ \pm 3^\circ$  C, and the ratio of the solid phases at this point is  $CaAl_2Si_2O_8$  37 :  $SiO_2$  63.

Schairer and Bowen (1947) found the

eutectic of the anhydrous system to be at  $1368^{\circ} \pm 2^{\circ}$  C and  $\text{CaAl}_2\text{Si}_2\text{O}_8$  50.5 :  $\text{SiO}_2$  49.5 weight per cent. The shift of this point toward increasing  $\text{SiO}_2$  at low  $\text{H}_2\text{O}$  pressures is a consequence of the greater lowering of the saturated liquidus of the silica polymorphs cristobalite and tridymite relative to that of  $\text{CaAl}_2\text{Si}_2\text{O}_8$ . A trend toward a lower  $\text{SiO}_2$  content at the four-phase point was anticipated at pressures higher than 2000 bars because at such pressures the rate of lowering of the saturated

conditions the proportion of quartz crystallizing from the liquid relative to feldspar crystals will decrease on that large portion of the liquidus where  $\text{CaAl}_2\text{Si}_2\text{O}_8$ -rich plagioclase is the first feldspar phase to appear with quartz.

When a silica mineral and feldspar are crystallizing simultaneously, the ratio of silica mineral to feldspar crystallizing will also vary with the  $\text{H}_2\text{O}$  pressure, increasing as the  $\text{H}_2\text{O}$  pressure increases to about 500 bars and decreasing as it increases

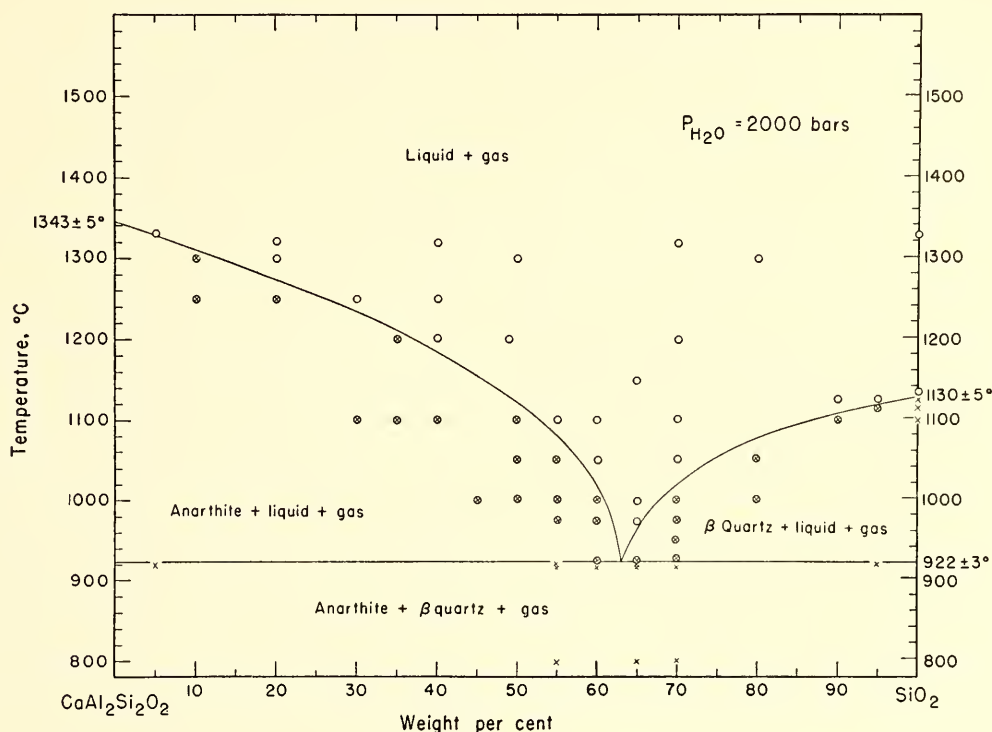


Fig. 48. Projection of the ternary system  $\text{CaAl}_2\text{Si}_2\text{O}_8$ - $\text{SiO}_2$ - $\text{H}_2\text{O}$  at 2000 bars  $\text{H}_2\text{O}$  pressure.

liquidus of anorthite with increasing  $\text{H}_2\text{O}$  pressure is greater than the rate of lowering of the saturated liquidus of quartz. Preliminary results on the four-phase point at 5000 bars indicate a shift toward a lower ratio of  $\text{SiO}_2$  and suggest that the ratio is close to An 42 :  $\text{SiO}_2$  58. A similar shift of the four-phase point toward  $\text{SiO}_2$  at low  $\text{H}_2\text{O}$  pressures and reversal at higher pressures was shown by Tuttle and Bowen in the systems  $\text{NaAlSi}_3\text{O}_8$ - $\text{SiO}_2$ - $\text{H}_2\text{O}$  and  $\text{KAlSi}_3\text{O}_8$ - $\text{SiO}_2$ - $\text{H}_2\text{O}$ . The ratio of  $\text{SiO}_2$  to  $\text{CaAl}_2\text{Si}_2\text{O}_8$  is greater than that for either  $\text{NaAlSi}_3\text{O}_8$  or  $\text{KAlSi}_3\text{O}_8$  at corresponding pressures. Accordingly, during isobaric crystallization under equilibrium

above this pressure. These relations suggest a possible geological barometer for  $\text{H}_2\text{O}$  pressure. The difference between the  $\text{SiO}_2$  content at the field boundary in the anhydrous system and the maximum ratio of  $\text{SiO}_2$  in the corresponding hydrous systems is of the order of 10 per cent, and should be easily detectable from the relative abundances of silica mineral and feldspar phenocrysts in lavas. In a sequence of flows of the same composition it may prove possible to use the variation of the ratio of these phenocrysts to each other as a measure of the  $\text{H}_2\text{O}$  pressure on the magma chamber where the phenocrysts formed, and to demonstrate a relation be-



tween the intensity of volcanic activity and  $H_2O$  pressure.

Another application is indicated by J. J. Norton, of the U. S. Geological Survey, through his detailed mineralogical studies of the Hugo pegmatite. This pegmatite contains quartz and feldspar in all its zones, and Norton's data indicate that starting from the wall zone the compositions of successive zones of the pegmatite, neglecting mica, first increase in  $SiO_2$  and then decrease. As the  $CaAl_2Si_2O_8$  content is low, these observations suggest that the  $H_2O$  pressure was changing, presumably increasing, during crystallization of the successive pegmatite zones.

If the effect of other components is neglected, the fact that granitic rocks in general plot near the silica-feldspar field boundary of the anhydrous "synthetic granite" system could be taken to mean that the liquids from which these granitic rocks crystallized formed at low  $H_2O$  pressures. This need not be so, however, as a similar position for this boundary also occurs at  $H_2O$  pressures between 2000 and 5000 bars, a value well within geologically probable limits. The  $SiO_2$  ratio apparently continues to decrease at higher pressures, so that crystallization of granitic rocks at very high  $H_2O$  pressures ( $>10,000$  bars) seems precluded.

Work in the system  $CaAl_2Si_2O_8-SiO_2-H_2O$  will include quantitative data on the  $H_2O$  content of the liquids formed and will indicate how this varies as the temperature and pressure change. The liquidus will be determined at 5000 and 10,000 bars. These data will be useful for quantitative aspects of the theory of the effects of  $H_2O$  in silicate systems.

#### OPTICAL PROPERTIES OF HEATED PLAGIOCLASES

*J. R. Smith*

Optical measurements continue to be the most widely used method of determining compositions of the ubiquitous plagioclase

classes. In view of the occurrence of different structural modifications of plagioclases in rocks, it is important to know what differences, if any, exist between the optical properties of the different modifications. In last year's report (Year Book 55), it was shown that the optic axial angle ( $2V$ ) of natural plagioclases throughout most of the composition range is changed significantly by heat treatment.  $2V$  is therefore a convenient measure of the structural state of a plagioclase. Measurements of the changes produced by heat treatment in refractive indices and in total birefringence have now been made. The methods of measurement, described in detail elsewhere (in press), are such as to give an estimated accuracy of  $\pm 0.0004$  for refractive indices and  $\pm 0.0002$  for total birefringence.

Ten samples of chemically analyzed natural plagioclases whose optical properties are accurately known were heated either in the dry way or hydrothermally until X-ray powder diffraction patterns showed them to be inverted to the "maximum" high-temperature forms. The optical properties were then measured again by the same methods. The data are shown in figure 49, which is largely self-explanatory; the most important point to note is that the difference in  $N_x$  between the high- and low-temperature forms is very slight or zero throughout the entire composition range, so that measurements of  $N_x$  will give a reliable estimate of the composition of a plagioclase regardless of its structural state. For plagioclases more calcic than  $An_{20}$ , any of the three refractive indices may be used. The composition being known, the structural state can be determined by the optical or X-ray methods described in previous Year Books (54, 55). Studies of natural plagioclases by these methods will undoubtedly aid in the interpretation of the crystallization and cooling history of many rocks.

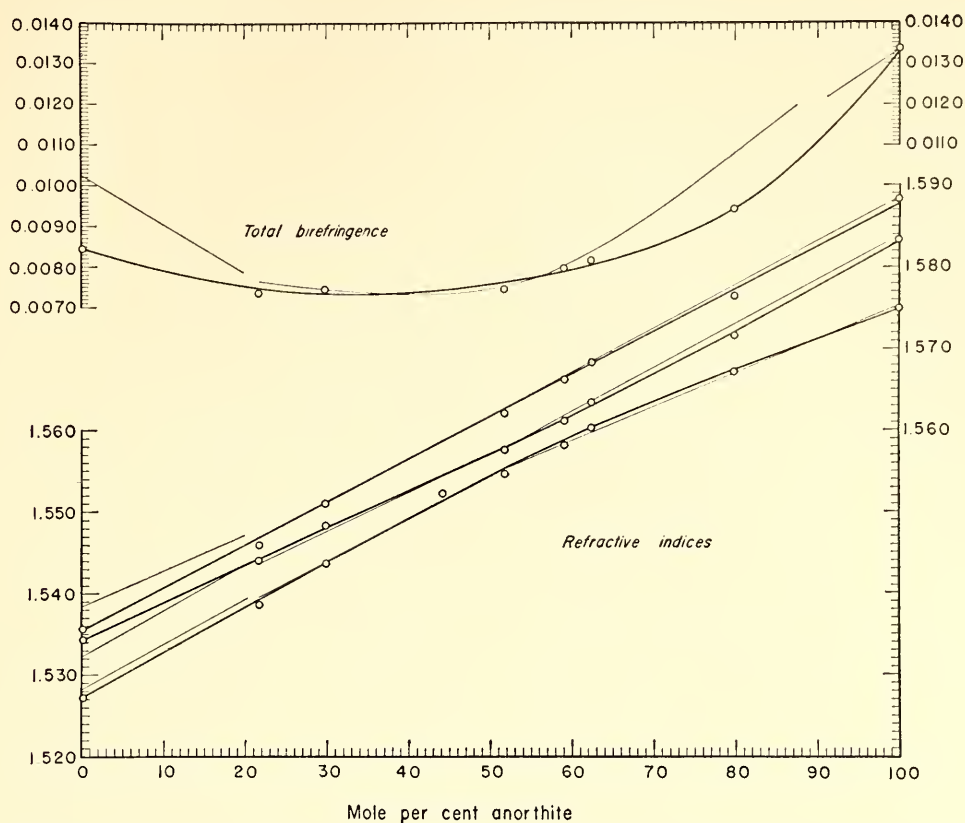


Fig. 49. Light lines are total birefringence and refractive indices of natural plagioclases from large plutonic intrusions. The circles and the heavy lines represent total birefringence and refractive indices of some of the same plagioclases after they had been inverted to the "maximum" high-temperature modifications by heating.

## THE CRYSTALLIZATION OF ROCK-FORMING MINERALS FROM MAGMAS AND THE NATURE OF THE RESIDUAL LIQUID

*J. F. Schairer*

All the important rock-forming minerals of the igneous rocks, with the exception of quartz, have a variable composition. They are solid solutions which undergo progressive changes in chemical composition during progressive cooling and crystallization of the magma. Even when there are no interruptions in the cooling cycle, there are continuous or discontinuous changes in the compositions of individual minerals and in the kinds of minerals crystallizing from the liquid phase. A mineral or assemblage of minerals stable at an early stage in the crystallization process may become unstable at a later stage and undergo transformation to new mineral assemblages with changes in both chemical composition and crystal system. Because of the very complexity of the mutual melting and stability relations, much informa-

tion can be gleaned from the nature of the minerals and mineral assemblages.

Two years ago (Year Book 54, pp. 141-142) we discussed the relations between early- and late-crystallizing minerals from melts and the nature of residual liquids from crystallization. During the past year we have acquired specific information on mineral assemblages, the direction of change of composition of the liquid phase during crystallization, and the nature of the residual liquid in a large part of the quaternary system  $\text{Na}_2\text{O}-\text{MgO}-\text{Al}_2\text{O}_3-\text{SiO}_2$ .

The three volumes albite-corundum-spinel-silica, albite-forsterite-cordierite-spinel, and albite-cordierite-spinel-silica constitute a large portion of the regular (equilateral) tetrahedron employed to describe the phase-equilibrium relations in the



quaternary system  $\text{Na}_2\text{O}-\text{MgO}-\text{Al}_2\text{O}_3-\text{SiO}_2$ . These volumes include that portion of the quaternary system of most interest to the geologist or petrologist who is concerned with the origin of igneous rocks or with the mineral assemblages of metamorphic rocks. All the crystalline phases found in these volumes are common rock-forming minerals or accessory minerals of either igneous or metamorphic rocks.

That portion of the quaternary system lying between albite, corundum, forsterite, spinel, and silica is being studied by a series of triangular joins. Some years ago at this Laboratory Greig made a reconnaissance of the system albite-forsterite-silica, a ternary system within this quaternary system (unpublished data of J. W. Greig). He located two ternary invariant points, albite + forsterite + magnesium metasilicate + liquid and albite + tridymite + magnesium metasilicate + liquid, which lie quite close in composition to the binary side line albite-silica. During the past year we prepared an extensive series of melts in three triangular joins; we present the data on them now.

In figures 50, 51, and 52, the phase-equilibrium data obtained by the method of quenching are presented graphically. In these figures, open double circles represent the compositions of chemical compounds, and black dots represent the compositions of mixtures studied. The diagrams are divided into areas by heavy curves. The areas labeled mullite, cordierite, spinel, albite, etc., are plane sections of phase volumes (with curved faces) within the tetrahedron in the join which the particular diagram represents. Each phase volume within the tetrahedron gives the compositions of all quaternary liquids (one liquid phase) in equilibrium with a single crystalline phase. The heavy curves on the joins are traces of the curved boundary surfaces between two adjacent primary phase volumes within the tetrahedron in the plane (join) which the particular diagram represents. Where three heavy curves meet at a point in the join, these points are

piercing points of curved lines within the tetrahedron in the plane (join) which the particular diagram represents. Along these curved lines, three curved surfaces (faces of adjacent primary phase volumes) intersect. Such a curved line is called a quaternary univariant line or quintuple line. Along such univariant lines three solid phases are in equilibrium with the liquids whose compositions lie on such lines. In figures 50, 51, and 52, light lines show contours of temperature (isotherms).

Figure 50 shows the phase-equilibrium relations in the join albite-cordierite-silica. This join cuts the phase volumes of mullite, cordierite, spinel, albite, tridymite, and cristobalite. Three univariant lines pierce this join at the points *C*, *G*, and *I*, respectively. The point *I* is a ternary eutectic, and is, therefore, a temperature maximum on the univariant line albite + cordierite + tridymite + liquid. The point *F* is the binary eutectic albite + cordierite + liquid. Mixtures whose compositions lie near the ternary eutectic are viscous and quite difficult to crystallize completely. They require runs of several weeks' duration to attain equilibrium between crystals and liquid. Experiments are still in progress to locate the position and temperature of point *I* more accurately.

Figure 51 shows the phase-equilibrium relations in the join albite-forsterite-cordierite. This join cuts the phase volumes of forsterite, albite, spinel, mullite, and cordierite. This last phase volume is cut twice by this join. Three univariant lines pierce this join at the points *N*, *O*, and *P*, respectively. The point *P* is a ternary eutectic and is, therefore, a temperature maximum on the univariant line albite + forsterite + cordierite + liquid. The point *Q* is the binary eutectic albite + forsterite + liquid.

Figure 52 shows the phase-equilibrium relations in the join albite-magnesium metasilicate-cordierite. This join cuts the phase volume forsterite, protoenstatite, albite, cordierite, spinel, and mullite. Three univariant lines pierce this join at the points *U*, *V*, and *W*, respectively.

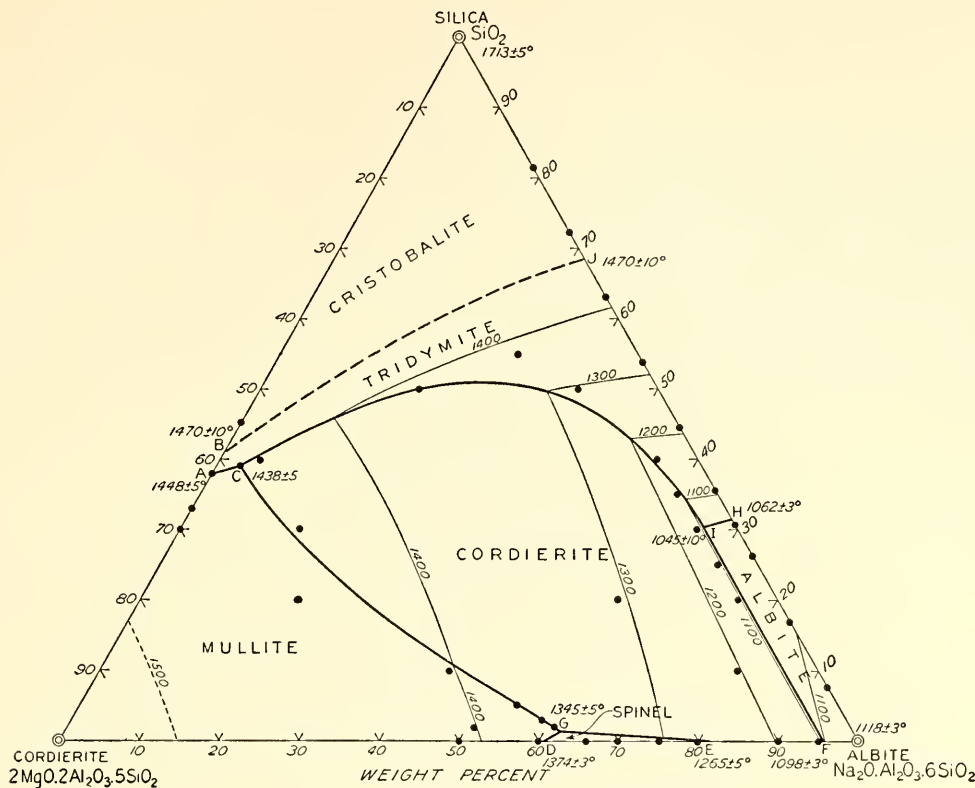


Fig. 50. Equilibrium diagram of the join albite-cordierite-silica, showing compositions studied, primary phase volumes cut by this join, piercing points of quaternary univariant lines, and isotherms.

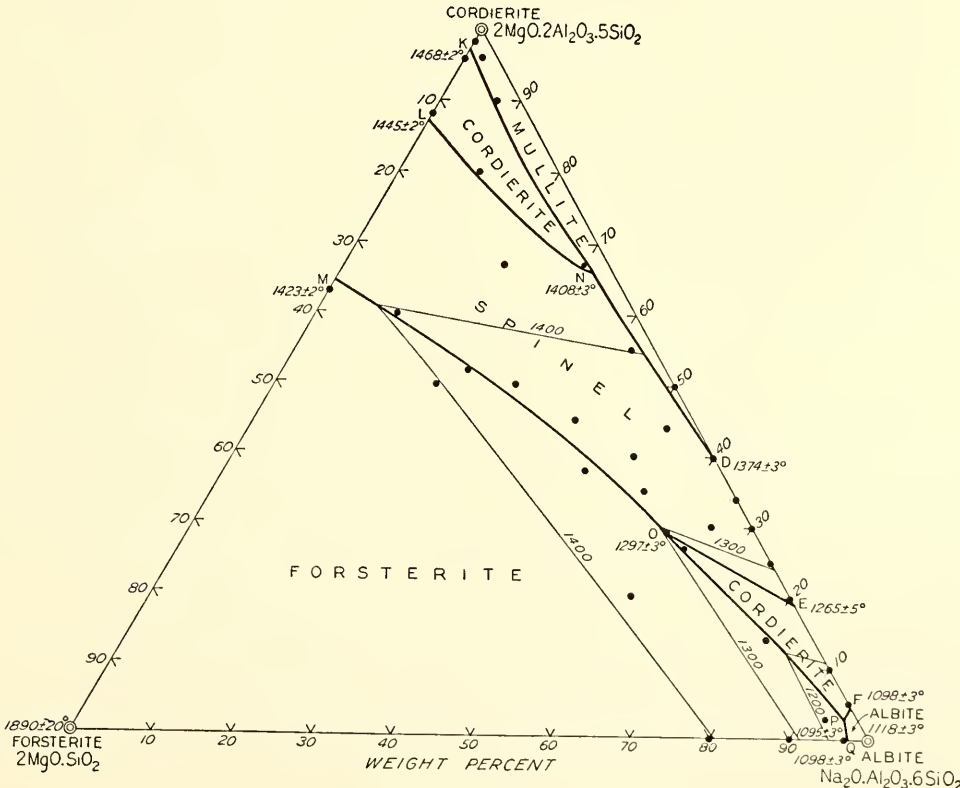


Fig. 51. Equilibrium diagram of the join albite-forsterite-cordierite.



The data just presented on three triangular joins indicate within approximate limits the temperatures and compositions of seven quaternary invariant points and describe the crystallization behavior of compositions in the volumes albite-cordierite-spinel-silica, albite-forsterite-cordierite-spinel, and albite-cordierite-spinel-silica. The relations between univariant lines and ternary and quaternary invariant points are shown diagrammatically in figure 53. All uni-

*A, B, C, and D* from the other quaternary invariant points and that *D* is the crystallization goal of the liquid in the volume albite-cordierite-spinel-silica; similarly, that the temperature maxima in *CE* and *EF* separate the quaternary invariant point *E* from the other quaternary invariant points and that *E* is the crystallization goal of the liquid in the volume albite-forsterite-cordierite-spinel; and, similarly, that the temperature maxima in *EF* and

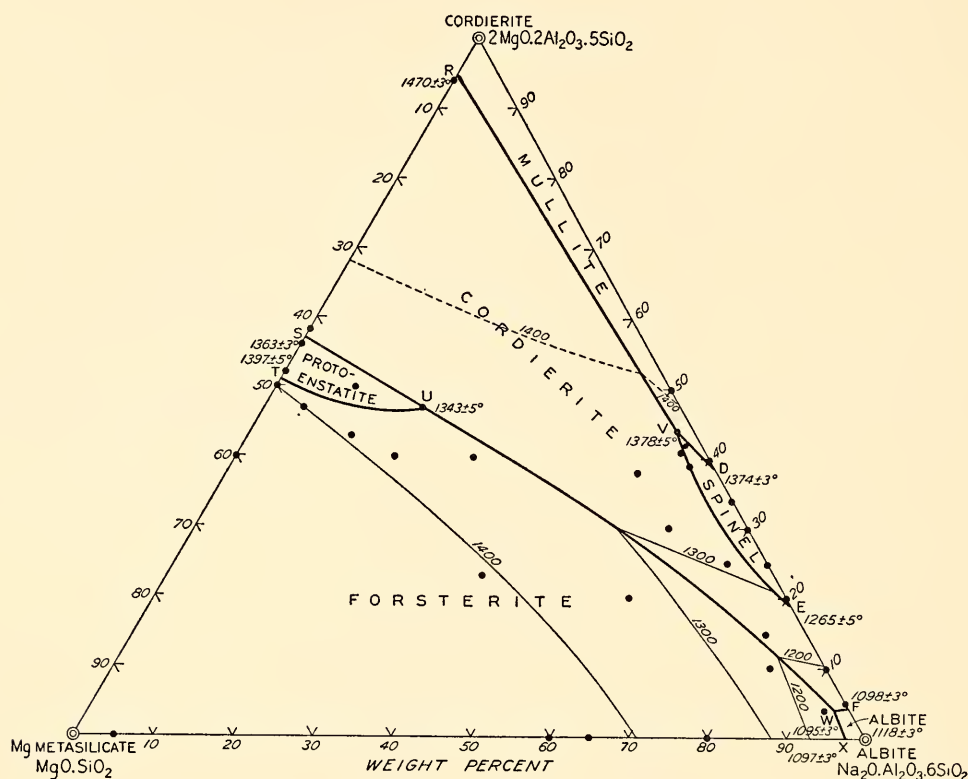


Fig. 52. Equilibrium diagram of the join albite-magnesium metasilicate-cordierite.

variant lines within the tetrahedron are curved lines. For simplicity, they are shown as straight lines in the figure, which is not spatial but merely depicts the relations of quaternary invariant points to one another and to certain ternary invariant points lying in a face of the tetrahedron. The lengths of the univariant lines in figure 53 are arbitrary and without significance. Arrows on the univariant lines indicate the direction of falling temperature.

An examination of figure 53 shows that the temperature maxima in *CE* and *DG* separate the quaternary invariant points

*DG* separate the quaternary invariant points *F* and *G* from the other quaternary invariant points and that *G* is the crystallization goal of the liquid in the volume albite-cordierite-spinel-silica.

An examination of figure 50 shows that the ternary eutectic albite + cordierite + tridymite + liquid (*I* of fig. 50) lies very close in temperature and composition to the binary eutectic albite + tridymite + liquid (*H* of fig. 50). An examination of figure 53 and a study of the tetrahedral model of the quaternary system shows that both *D* and *G* (fig. 53) must also lie close in temperature and composition to each other



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and to the ternary eutectic albite + cordierite + liquid. Thus we see that during crystallization all compositions in two large volumes albite-cordierite-spinel-silica and albite-cordierite-spinel-silica proceed toward a similar goal for the composition of the residual liquid. This goal is a soda granite in composition in these potash- and lime-free mixtures.

Some of the liquids in the two volumes just discussed are not too far removed from possible magmas. The "simplified magmas" in  $\text{Na}_2\text{O}-\text{MgO}-\text{Al}_2\text{O}_3-\text{SiO}_2$  are, of course, anhydrous instead of having at least a small water content. There is only soda present with potash lacking, only magnesia present with ferrous oxide lacking, and only alumina present with ferric oxide lacking; there is no lime. In spite

of these deficiencies, it can be seen that a large range of rock-forming compositions would give a common end product of crystallization, particularly if differentiation proceeded by fractional crystallization. Note the significant fact that this same end product, a soda granite, would be reached even if these simplified magmas were contaminated with numerous small fragmental xenoliths of basic rocks such as peridotites or dunites, or if they were contaminated by assimilation, in whole or in part, with xenoliths of highly aluminous sediments.

Thus these studies of soda-bearing melts add further quantitative evidence to support Bowen's arguments for the importance in petrogenesis of  $\text{KAlSiO}_4-\text{NaAlSiO}_4-\text{SiO}_2$  as "petrogeny's residua system."

## GRANITIC PEGMATITES

*P. M. Orville*

The origin of granitic pegmatites and the unusual textures and structures within them have been the subject of geological investigation and speculation for many years. Recent laboratory investigations in the  $\text{Ab}-\text{Or}-\text{SiO}_2-\text{H}_2\text{O}$  ("synthetic granite") system enable the course of crystallization in melts which closely approximate the granitic pegmatites in composition to be considered on a quantitative basis.

Pegmatites enriched in potassic feldspar in their upper portions are common in the Black Hills, South Dakota; Pala, California; Colorado; New England; and the southern Appalachian states. In 1954 at Yale University a study of a complex of layered pegmatites in the southern Black Hills, South Dakota, showing such a distribution of potassic feldspar, was begun in the hope that the field and laboratory data together might lead to an understanding of the process by which this segregation took place.

The pegmatites studied are thin, steeply tabular bodies separated parallel to their median plane into an upper unit contain-

ing large perthitic microcline crystals set in a fine-grained plagioclase-quartz matrix and a lower unit consisting of plagioclase-quartz-muscovite aplite. Such sharply layered pegmatite dikes are gradational into dikes having a uniform composition and aplitic texture from wall to wall and a mineralogic and bulk chemical composition identical with that of the layered pegmatites.

The bulk compositions of the pegmatites (neglecting muscovite) fall on, or very near, the field boundary between the quartz and alkali feldspar fields approximately halfway between the "granitic minimum" composition and the  $\text{Ab}-\text{SiO}_2$  side line of the liquidus in the system  $\text{Ab}-\text{Or}-\text{SiO}_2-\text{H}_2\text{O}$  at 2000 bars. The composition of the upper unit corresponds closely to the composition of the minimum in the synthetic granite system at this pressure, and the composition of the lower unit falls near the  $\text{Ab}-\text{SiO}_2$  side line on the quartz-alkali feldspar field boundary.

The presence of a few per cent An in the melt raises the crest of the ternary feldspar solvus by a large amount, and the solvus

intersects the liquidus within a short distance of the Or–Ab side line (Yoder, Stewart, and Smith, this report). The plagioclase from these pegmatites is a calcic albite ( $\text{Ab}_{93}\text{An}_7$ ), and this amount of An may be sufficient so that crystallization of two feldspars takes place from the magma.

It is possible that the separation of these pegmatites into two compositional units

is the result of fractional crystallization of a hydrous silicate melt. The aplitic unit might represent the first stage of crystallization as quartz and albite crystallize together along the quartz-plagioclase field boundary. The coarse-grained unit containing potassic feldspar might represent the crystallization of the rest liquid that approaches the composition of the synthetic granite minimum.

## PYROXENES

*J. F. Schairer and F. R. Boyd, Jr.*

For a petrologist concerned with the genesis of igneous and high-grade metamorphic rocks there is no more important system than the pyroxene quadrilateral  $\text{MgSiO}_3$ – $\text{CaMgSi}_2\text{O}_6$ – $\text{CaFeSi}_2\text{O}_6$ – $\text{FeSiO}_3$ . Pyroxenes whose compositions (neglecting minor components) lie in this quadrilateral are major constituents of basalts, gabbros, andesites, and important groups of metamorphic rocks such as the iron formations. Rhyolites often contain iron-rich pyroxenes in minor, though genetically significant, amounts. The pyroxenes also form monomineralic and bimineralic aggregates in the pyroxenites and peridotites. Laboratory study of the pyroxenes will not only provide quantitative data about the physical conditions under which these rocks have formed but will also give us a better understanding of the courses of chemical fractionation in magmas.

The importance of pyroxenes has long been recognized by experimental petrologists. The melting relations along the join  $\text{MgSiO}_3$ – $\text{CaMgSi}_2\text{O}_6$  were first studied at this Laboratory in 1914. The joins  $\text{CaFeSi}_2\text{O}_6$ – $\text{FeSiO}_3$  and  $\text{MgSiO}_3$ – $\text{FeSiO}_3$  have been studied more recently. Investigations along the side lines of the quadrilateral  $\text{MgSiO}_3$ – $\text{CaMgSi}_2\text{O}_6$ – $\text{CaFeSi}_2\text{O}_6$ – $\text{FeSiO}_3$  have yielded data of vital interest, but inasmuch as most natural pyroxene compositions fall well within the quadrilateral the major gains have yet to be made.

It is our intention to carry through a study of both the melting and subsolidus equilibria in the entire quadrilateral. The

data discussed below, on the join  $\text{MgSiO}_3$ – $\text{CaMgSi}_2\text{O}_6$ , represent our initial effort in what will be a continuing program in coming years.

### THE JOIN $\text{MgSiO}_3$ – $\text{CaMgSi}_2\text{O}_6$

*F. R. Boyd, Jr., and J. F. Schairer*

The crystal-liquid equilibria along the join  $\text{MgSiO}_3$ – $\text{CaMgSi}_2\text{O}_6$  were determined many years ago by Bowen (1914) in his study of the system forsterite–diopside–silica. Bowen's work has been checked in detail and found to be substantially accurate. We have determined liquidus temperatures on compositions between  $\text{MgSiO}_3$  and  $\text{CaMgSi}_2\text{O}_6$  prepared at 5 weight per cent intervals. All our data lie precisely on the curves of Bowen. For those compositions with a forsterite liquidus we have determined the temperature of appearance of pyroxene. These data are in agreement with those interpolated from Bowen's data. The beginning of melting was determined for all these compositions, and the results check those of Bowen closely.

Atlas (1952) has investigated the subsolidus region in the system  $\text{MgSiO}_3$ – $\text{CaMgSi}_2\text{O}_6$  using a lithium fluoride flux. Our work confirms that of Atlas on the existence and approximate position of the solvus. Our locations of the solvus curves differ from those in the Atlas diagram largely in that we have found that the solvus intersects the solidus; i.e., there is not a complete solid solution between  $\text{MgSiO}_3$  and  $\text{CaMgSi}_2\text{O}_6$ .



Our work on the join  $\text{MgSiO}_3$ – $\text{CaMgSi}_2\text{O}_6$  is not yet finished. We have presented in figure 54 only those portions of the subsolidus diagram that we think are unlikely to undergo any further modification. Our principal uncertainty remains the relations involving the various polymorphs of  $\text{MgSiO}_3$ .

We have used a variety of techniques in determining the subsolidus phase rela-

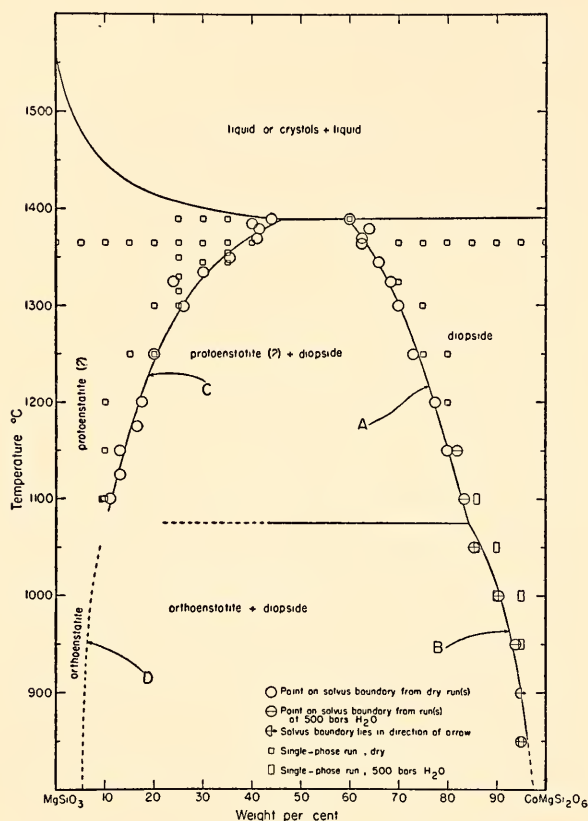


Fig. 54. Subsolidus diagram of the system  $\text{MgSiO}_3$ – $\text{CaMgSi}_2\text{O}_6$ .

tions along this join. Runs on the solvus curves (A, B, C, and D in fig. 54) have been made dry in the temperature range between  $1100^\circ$  and  $1400^\circ$  C. Runs have been made in hydrothermal quenching apparatus from a temperature of  $1150^\circ$  C down to about  $850^\circ$  C. We have also used a heating stage on an X-ray diffractometer up to a temperature of about  $1350^\circ$  C.

Our starting material for both dry and hydrothermal runs has generally been glass. We have, however, been able to unmix homogeneous, crystalline phases (prepared at temperatures just below the solidus temperature) at temperatures down

to about  $1200^\circ$  C on the diopside side of the solvus and down to about  $1100^\circ$  C on the enstatite side.

The determinations of compositions of phases along the solvus curves have been made by X-ray methods. We have developed four curves relating X-ray spacing to composition in this system. Two of these, which have proved to be superior in practice and have been used almost exclusively, are presented in figures 55 and 56.

Figure 55 shows the variation in spacing of the  $31\bar{1}$  peak in diopside as a function of composition. This curve is linear be-

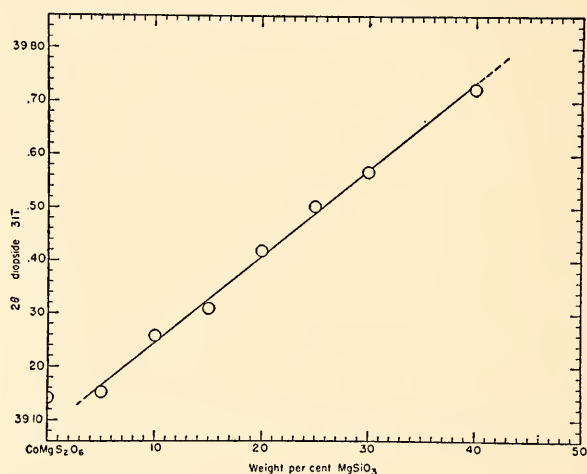


Fig. 55. Variation of the  $31\bar{1}$  spacing of diopside with composition in the system  $\text{MgSiO}_3$ – $\text{CaMgSi}_2\text{O}_6$ .

tween  $\text{Di}_{95}\text{En}_5$  and  $\text{Di}_{60}\text{En}_{40}$ . Between  $\text{Di}_{95}\text{En}_5$  and pure diopside there is an abrupt change in slope, which does not appear to be an inversion since the X-ray parameters of both pure diopside and  $\text{Di}_{95}\text{En}_5$  prepared hydrothermally at about  $900^\circ$  C show no significant differences from the parameters of samples prepared dry above  $1300^\circ$  C. The solvus curve ceases to be of interest in the composition region between  $\text{Di}_{95}\text{En}_5$  and pure diopside; accordingly, we have not further pursued the matter of this break in slope.

Figure 56 shows the variation in spacing with composition of a clinoenstatite peak at about  $57^\circ 2\theta$   $\text{CuK}\alpha$ . This spacing has been used to determine the position of the solvus curve C.

Points on the solvus boundaries deter-

mined with the aid of these X-ray curves are plotted as circles in figure 54: open circles represent data from dry runs; circles with a horizontal bar, data from hydrothermal runs at 500 bars ( $\text{H}_2\text{O}$ ). Hydrothermal data and dry data overlap at  $1150^\circ\text{C}$  on curve *A*. The agreement is within experimental error. Along curves *A* and *B* each point represents an average of up to five runs using, generally, two or more

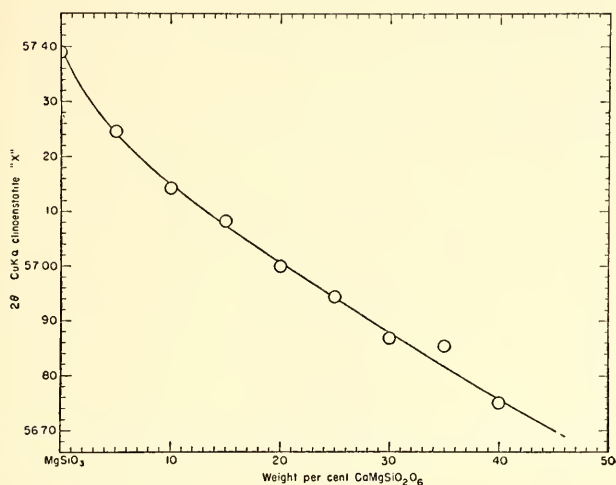


Fig. 56. Variation of a clinoenstatite spacing with composition in the system  $\text{MgSiO}_3$ - $\text{CaMgSi}_2\text{O}_6$ .

bulk compositions. The spread of data at a given temperature is seldom more than 2 per cent in composition. Along curve *C* we have had trouble with X-ray interference from the coexisting diopside phase, and only bulk compositions in the two-phase region closest to the solvus curve were used to fix its composition.

We have checked our locations of the solvus curves by making runs in the single-phase regions close to the curves over a wide range in temperature. These runs are indicated in figure 54 by squares or rectangles. The agreement in X-ray spac-

ing of these runs with those made at  $1365^\circ\text{C}$ , just below the solidus, has been within X-ray error (about  $\pm 0.02^\circ 2\theta$ ).

There is a pronounced change in slope of the solvus boundary on the diopside side at about  $1075^\circ\text{C}$ . It is probably produced by the inversion of orthoenstatite to a higher-temperature form.

We have not yet established a determinative curve for orthoenstatite solid solutions. In the temperature range below  $1050^\circ\text{C}$ , however, diopside appears in our hydrothermal runs whose composition is richer in  $\text{CaMgSi}_2\text{O}_6$  than  $\text{En}_{90}\text{Di}_{10}$ . Curve *D* must, therefore, lie approximately as shown.

We are not yet sure of the stable crystalline form of the pyroxene in the single-phase region bordering curve *C*. In dry runs bordering curve *C* we have generally obtained clinoenstatite; in hydrothermal runs (all below  $1100^\circ\text{C}$ ) we obtain protoenstatite. We have located curve *C* by means of the clinoenstatite spacing in figure 56. Whatever form of enstatite turns out to be stable along curve *C*, it is improbable that the position of the solvus curve itself will have to be modified. The inversions involving clino- and protoenstatite are very rapid, whereas the unmixing reaction is relatively sluggish. Hence, it is most unlikely that any unmixing takes place during inversion in the quench.

In our hydrothermal studies on pure  $\text{MgSiO}_3$  we have prepared well crystallized orthoenstatite and protoenstatite. Unlike protoenstatite prepared dry, the hydrothermal material shows little tendency to invert to clinoenstatite. We shall withhold our detailed results on this aspect of the problem, however, until we are sure of the stable fields of these various polymorphs.

## CHLORITOID

*L. B. Halferdahl*

Chloritoid is a geologically significant mineral which has been described from many occurrences, and yet its properties and composition are still disputed among

mineralogists. Many petrologists believe that it can form only under restricted conditions, notably those in which stress plays a part, despite the fact that its growth has



been recorded in other geological environments. Some preliminary results of an investigation of the chemical composition, the optical and X-ray properties, the stability, and the nature of the occurrences of chloritoid were reported last year. Additional results are given here.

Five new chemical analyses of chloritoid, including one so-called "ottrelite" from Salm Château, Belgium, and all the other available analyses that are considered reliable indicate that the composition of chloritoid can be represented by the general formula  $\text{FeO} \cdot \text{Al}_2\text{O}_3 \cdot \text{SiO}_2 \cdot \text{H}_2\text{O}$  with the following ranges in the amounts of substitutions now on record:

Substitution	Atomic Per Cent
Mg $\rightarrow$ Fe''	0 to 40
Mn $\rightarrow$ Fe''	0 to 17
Fe''' $\rightarrow$ Al	0 to 10
F $\rightarrow$ OH	Less than 0.25
Fe''' $\rightarrow$ Fe''H	Small
2Fe''' $\rightarrow$ 3Fe''	Very small

It is important to realize that these may not represent the maximum possible amounts of substitutions. Nevertheless, the purity of the chloritoid used in all previously published analyses showing more than this amount of manganese is subject to question.

X-ray investigations have revealed two chloritoid polymorphs with the unit cell parameters given in table 23. On a geometrical basis it is possible to consider the unit cell of the structure of monoclinic chloritoid determined by Harrison and Brindley (1957) as consisting of two triclinic unit cells in a zigzag arrangement. Such cells have parameters very similar to those of the triclinic chloritoid from Chibougamau, Quebec. This structural relationship is similar to that of the clino- and orthopyroxenes.

Measurements made on the universal stage show that the orientation of the optical indicatrix varies considerably in different grains of the same chloritoid sample. In triclinic chloritoid no principal optic direction is required by symmetry to lie in (001). Because of the variations in the

orientation of the optical indicatrix, however, one or even two principal optic directions may chance to lie in (001) with neither coinciding with the *b* axis. Therefore, an observation that one principal optic direction lies in (001) may not be sufficient to establish monoclinic symmetry for the chloritoid grain in question, particularly if no principal optic direction lies in (001) in other grains.

Optical measurements made on samples of chloritoid from 11 localities including both triclinic and monoclinic polymorphs show that *Z* makes angles varying from 2° to 30° with the normal to (001). In

TABLE 23. Unit Cell Parameters of Chloritoid Polymorphs

Locality	Üstüaçiksarinç, Turkey	Chibougamau, Quebec
Polymorph	Monoclinic	Triclinic
<i>a</i> .....	9.48	9.50
<i>b</i> .....	5.48	5.48
<i>c</i> .....	18.19	9.16
$\alpha$ .....	90° 0'	96° 53'
$\beta$ .....	101° 46'	101° 49'
$\gamma$ .....	90° 0'	90° 2'
<i>G</i> (meas.) ..	3.79	3.79
<i>G</i> (calc.) ...	3.80	3.79

the monoclinic polymorphs measured,  $X=b$ . In the triclinic polymorphs, *X* and *Y* make angles from 0° to 30° with (001), but in many *Y* is closer to (001) than *X*. The orientation of *X* and *Y* with respect to the *a* and *b* axes in triclinic chloritoids was not determined, because no crystallographic directions other than the (001) cleavage were identified with certainty in the thin section and grain mounts of the specimens studied. In chloritoids from 11 localities the optic angle about *Z* ranges from 36° to 125°. Most, however, are optically positive with  $2V_Z$  in the range 45° to 60°. The refractive indices of chloritoid measured in this study and those previously published show that  $\alpha$  varies from 1.713 to 1.730,  $\beta$  from 1.719 to 1.734, and  $\gamma$  from 1.723 to 1.740. All indices de-

crease with increasing magnesium content of the chloritoids.

The results of hydrothermal experiments on natural chloritoid and on some oxide mixtures of the chloritoid composition are presented in figure 57. Because of the slug-

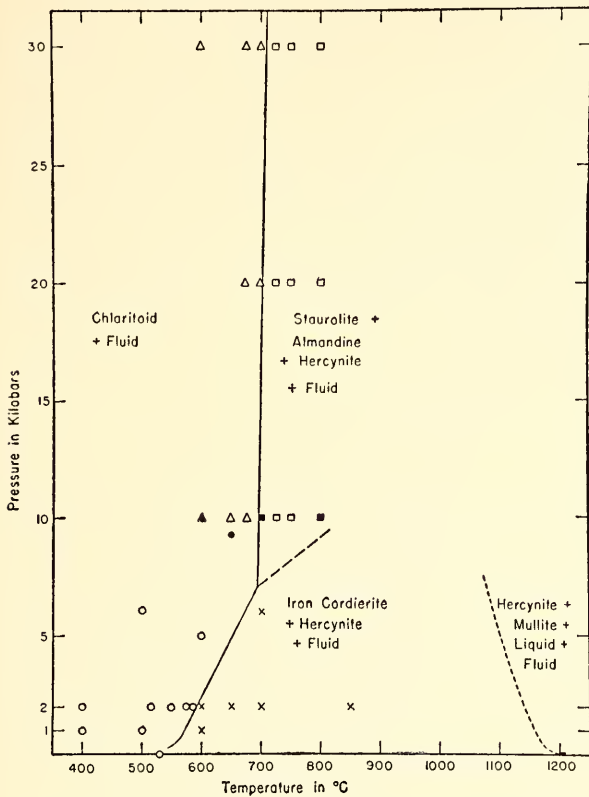


Fig. 57. Preliminary univariant curves for the reactions chloritoid  $\rightleftharpoons$  iron cordierite + hercynite + fluid (solid lines), chloritoid  $\rightleftharpoons$  almandine + staurolite + hercynite + fluid (solid lines), iron cordierite + hercynite + fluid  $\rightleftharpoons$  staurolite + almandine (long dashes), and iron cordierite + fluid  $\rightleftharpoons$  mullite + liquid + fluid (short dashes).

#### Explanation of Symbols

*Runs made in sealed platinum tubes and one in an evacuated glass tube:* Solid circle, chloritoid synthesized from mixes of oxides. Open circle, natural chloritoid did not change. Cross, natural chloritoid broke down to iron cordierite, hercynite, and fluid; iron cordierite + hercynite synthesized from oxide mixes. Solid square, natural chloritoid broke down to almandine, staurolite, hercynite, and fluid; almandine + staurolite + hercynite synthesized from oxide mixes.

*Runs made in co-operation with Boyd and England in squeezer apparatus built by them:* Open triangle, natural chloritoid persisted. Open square, natural chloritoid broke down to almandine, staurolite, hercynite, and fluid.

Solid rectangle, point determined by quenching (Schairer and Yagi, 1952).

gishness of the reactions at pressures below 10,000 bars and the difficulty of controlling the state of oxidation, these results must be regarded as preliminary. Nevertheless, they do provide an acceptable alternative to the widely held belief that the action of stress at any particular temperature and composition determines the mineral associations in regional metamorphism. This alternative is that the differences in mineralogy between regionally metamorphosed rocks and those formed in contact aureoles may be the result of different pressures prevailing in the two environments. Thus, in quartz-bearing rocks containing chloritoid, the reaction chloritoid + quartz  $\rightleftharpoons$  iron cordierite + fluid might be expected in contact metamorphism, and the reaction chloritoid + quartz  $\rightleftharpoons$  almandine + staurolite + fluid in regional metamorphism. Chloritoid-cordierite associations are known in the Vredefort Dome area of South Africa and the Santa Monica Mountains of California. Chloritoid-almandine-staurolite associations are known from many places in the Alps and from Unst in the Shetland Islands. At intermediate pressures the association staurolite-cordierite might be expected. Such associations are known from the Lizard area of Cornwall and from the contact aureole of the Bushveld complex in South Africa.

The conditions under which one of the chloritoid polymorphs will form in preference to the other are still unknown. The differences in energy required to produce one in place of the other are probably very small, and, hence, could be detected only with very great difficulty by the methods at present available. Natural occurrences, however, do provide some clues. Chloritoids obtained from hydrothermal veins and from hydrothermally altered rocks have been found to be triclinic. Chloritoids from regionally metamorphosed rocks have been found to be monoclinic or triclinic or both. Chloritoids associated with almandine, kyanite, or staurolite are monoclinic. This information may suggest that the



monoclinic polymorph forms more readily than the triclinic under severe conditions of pressure and temperature, or under more moderate conditions prevailing for a long time.

Studies of chloritoid-bearing rocks, published reports of chloritoid occurrences, and phase-rule considerations indicate that chloritoid can exist under certain conditions in equilibrium with one or more of the following minerals: quartz, chlorite, muscovite, magnetite, hematite, ilmenite, rutile, paragonite, almandine, staurolite, cordierite, biotite, pyrophyllite, kaolinite, kyanite, andalusite, and possibly sillimanite, corundum, diaspore, glaucophane, albite,

anorthite, epidote, zoisite, margarite, calcite, and siderite. The most common mineral assemblage in chloritoid-bearing rocks is chloritoid-quartz-chlorite-muscovite-rutile-iron oxide, the iron oxide being one or more of magnetite, hematite, ilmenite. These mineral associations and the environment in which they occur—regional metamorphism, contact metamorphism, hydrothermal veins and hydrothermally altered rocks, emery deposits—give further support to the suggestion that stress plays no greater part in the formation of chloritoid than in the formation of such minerals as muscovite, andalusite, pyroxene, or feldspar.

## ALKALI AMPHIBOLES

*W. G. Ernst*

Alkali amphiboles, one of the three principal groups of amphiboles, occur in a wide variety of rock types. Riebeckite is an important constituent of alkalic, silicic igneous rocks; it has also been described as a low-temperature authigenic mineral. Glaucophane and crossite (an intermediate member of the riebeckite-glaucophane series) are abundant in certain metamorphic rocks.

Hydrothermal study of the riebeckite-glaucophane series was initiated in 1956; preliminary data are now available. Magnesian riebeckite,  $\text{Na}_2\text{Mg}_3\text{Fe}_2^{+3}\text{Si}_8\text{O}_{22}(\text{OH})_2$ , and ferrous riebeckite,  $\text{Na}_2\text{Fe}_3^{+2}\text{Fe}_2^{+3}\text{Si}_8\text{O}_{22}(\text{OH})_2$ , have been synthesized, and work on these minerals as well as on magnesian glaucophane,  $\text{Na}_2\text{Mg}_3\text{Al}_2\text{Si}_8\text{O}_{22}(\text{OH})_2$ , is in progress.

With the exception of magnesian glaucophane, all compositions investigated contain iron capable of existing in two oxidation states; therefore the physical parameters governing stability include the partial pressure of oxygen as well as the temperature and total ( $\text{H}_2\text{O}$ ) pressure. Accordingly, in experiments employing iron-bearing minerals the  $P_{\text{O}_2}$  was fixed using buffers as described by Eugster.

*Magnesian riebeckite.* Preliminary

$P_{\text{H}_2\text{O}}-T$  stability diagrams for  $\text{Na}_2\text{Mg}_3\text{Fe}_2^{+3}\text{Si}_8\text{O}_{22}(\text{OH})_2$  using several  $P_{\text{O}_2}$  buffers are presented in figures 58, 59, and 60. Each diagram represents the intersections of boundary surfaces occurring within the  $P_{\text{H}_2\text{O}}-T-P_{\text{O}_2}$  volume with the surface defined by a specific buffer, and projected along the  $P_{\text{O}_2}$  axis (compare fig. 13 with fig. 14).

All three riebeckite diagrams exhibit the same general sequence of phases. The form of the riebeckite stability field is similar to that of other hydrous minerals. At low  $T$  and at a  $P_{\text{H}_2\text{O}}$  too low to form riebeckite, the stable assemblage consists of one or two iron oxides, an olivine,  $\text{Na}_2\text{O} \cdot 2\text{MgO} \cdot 6\text{SiO}_2$ ,  $\text{Na}_2\text{O} \cdot 5\text{MgO} \cdot 12\text{SiO}_2$  (compounds previously reported in the system  $\text{Na}_2\text{O}-\text{MgO}-\text{SiO}_2$  by Schairer, Yoder, and Keene), and vapor, except for the magnetite-hematite diagram, where acmite takes the place of  $\text{Na}_2\text{O} \cdot 2\text{MgO} \cdot 6\text{SiO}_2$ . At slightly higher temperatures, the assemblage consists of one or two iron oxides,  $\text{Na}_2\text{O} \cdot 5\text{MgO} \cdot 12\text{SiO}_2$ , an olivine, liquid, and vapor. In a still higher temperature range the  $\text{Na}_2\text{O} \cdot 5\text{MgO} \cdot 12\text{SiO}_2$  melts incongruently to orthopyroxene and liquid, and the assemblage then becomes one or two iron oxides, an olivine, an orthopyroxene, liquid, and vapor.

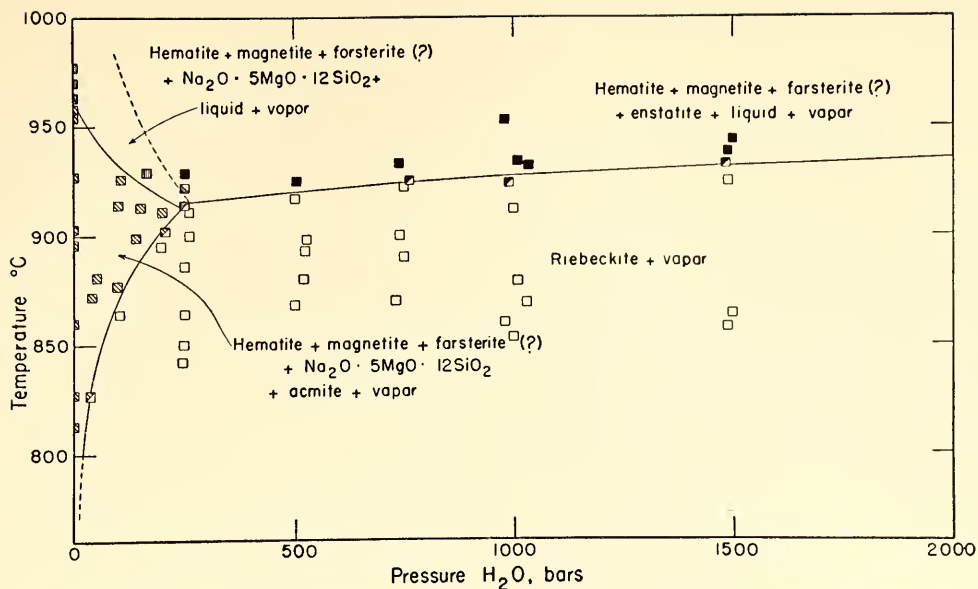


Fig. 58.  $P_{H_2O}$ - $T$  diagram for magnesian riebeckite using the hematite-magnetite buffer.

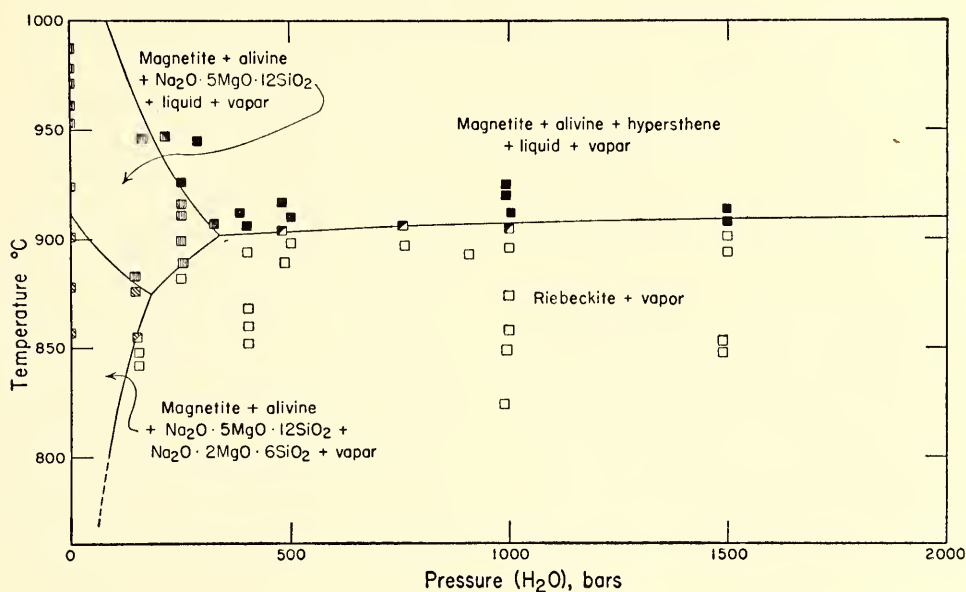


Fig. 59.  $P_{H_2O}$ - $T$  diagram for magnesian riebeckite using the magnetite-fayalite-quartz buffer.

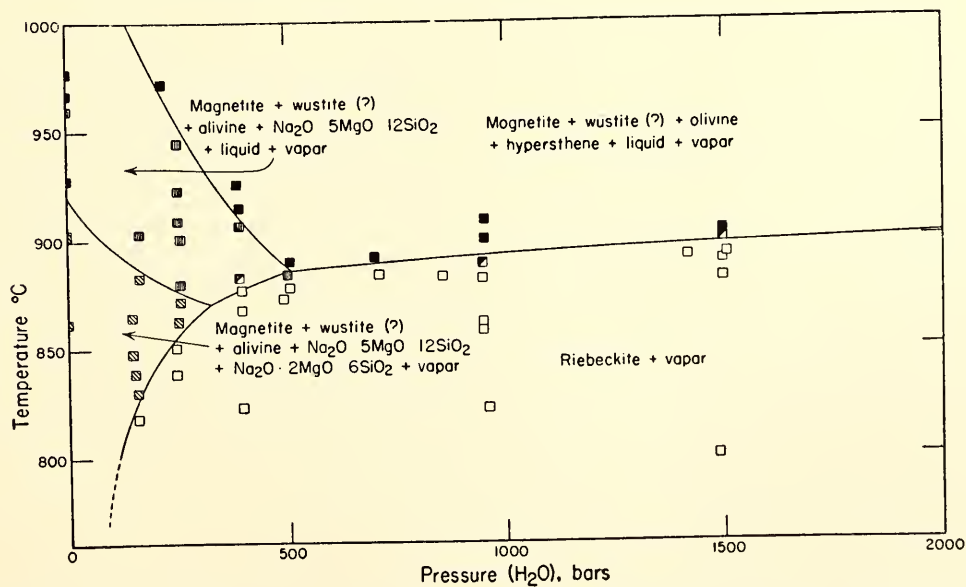


Fig. 60.  $P_{H_2O}$ - $T$  diagram for magnesian riebeckite using the magnetite-wüstite buffer.



The upper stability limit of riebeckite at a given  $P_{\text{tot}}$  is elevated by increasing the partial pressure of oxygen. The iron in the amphibole is predominantly ferric, and much of the iron is bivalent in the high-temperature phases. During decomposition there is an evolution of free oxygen. The evolution of oxygen results in a volume increase, so increased oxygen partial pressure tends to drive the reaction toward the assemblage of smaller volume (riebeckite).

All phases containing MgO also contain some FeO, the amount depending on the bulk composition and  $P_{\text{O}_2}$  as well as on the temperature and total pressure. Riebeckite contains some ferrous iron, too, and breaks down over a small temperature interval (approximately  $20^\circ$ ). For this reason, curves bounding the riebeckite fields in figures 58, 59, and 60 define the highest temperature at which amphibole is stable.

$\text{Na}_2\text{O} \cdot 5\text{MgO} \cdot 12\text{SiO}_2$  and  $\text{Na}_2\text{O} \cdot 2\text{MgO} \cdot 6\text{SiO}_2$  apparently increase their upper stability limits as the partial pressure of oxygen declines. Some ferrous iron is probably incorporated in these minerals. Oxygen in equilibrium with these compounds is used up during breakdown (an oxidation process in regard to the liquid and solid

phases). Hence a diminished  $P_{\text{O}_2}$  tends to increase the stability field of the large-volume assemblage (sodic silicate plus oxygen).

The experimental formation of riebeckite in equilibrium with a melt rich in  $\text{Na}_2\text{O}$  and  $\text{SiO}_2$  agrees with natural occurrences where riebeckite appears as a magmatic mineral in certain alkalic, silicic intrusives.

*Ferrous riebeckite.* Exploratory runs on the composition  $\text{Na}_2\text{Fe}_3^{+2}\text{Fe}_2^{+3}\text{Si}_8\text{O}_{22}(\text{OH})_2$  using a fayalite-magnetite-quartz buffer indicate that ferrous riebeckite breaks down at a temperature approximately  $150^\circ$  lower than the breakdown temperature of its magnesian analogue. Decomposition products below about 1500 bars  $P_{\text{H}_2\text{O}}$  are fayalite, acmite, magnetite, quartz, and vapor; above this pressure riebeckite melts incongruently to fayalite, magnetite, quartz, liquid, and vapor.

*Magnesian glaucophane.* Reconnaissance runs on the composition  $\text{Na}_2\text{Mg}_3\text{Al}_2\text{Si}_8\text{O}_{22}(\text{OH})_2$  have yielded an amphibole whose upper stability limit is  $20^\circ$  to  $80^\circ$  lower than that of magnesian riebeckite. The high-temperature assemblages include forsterite, albite, enstatite (?), liquid, and vapor.

## RECONNAISSANCE IN THE SYSTEM $\text{FeO}-\text{Fe}_2\text{O}_3-\text{SiO}_2-\text{H}_2\text{O}$

*J. R. Smith*

The problem of the origin and subsequent enrichment and metamorphism of iron ores of the Lake Superior type continues to be of great importance to economic geologists and petrologists. The bulk compositions of many of the ores and related rocks lie close to the quaternary system  $\text{FeO}-\text{Fe}_2\text{O}_3-\text{SiO}_2-\text{H}_2\text{O}$ . Experimental studies in the  $\text{FeO}-\text{Fe}_2\text{O}_3$  binary system have already contributed to our understanding of the conditions under which the iron oxides might have formed, but little is known of the stability relations of ternary and quaternary compounds in the quaternary system, largely because of the experimental difficulties involved in

controlling the partial pressures of oxygen in the presence of water under pressure. The technique developed by Eugster of using as buffers polyphase assemblages for which the temperature versus partial pressure of oxygen equilibrium relations are known offered a means of investigating portions of the system. It was planned to investigate first the stability fields of minnesotaite ( $3\text{FeO} \cdot 4\text{SiO}_2 \cdot \text{H}_2\text{O}$ ) and greenalite ( $3\text{FeO} \cdot 2\text{SiO}_2 \cdot 2\text{H}_2\text{O}$ ), both of which occur with the iron ores of the Lake Superior region. Knowledge of the stability fields of these minerals in terms of partial pressure of oxygen as well as of temperature and water pressure would provide

further evidence of the conditions existing during the formation and later history of the ores.

In attempts to synthesize minnesotaite and greenalite, over 100 experiments have been made at temperatures from 250° to 600° C, at total water pressures from 500 to 30,000 psi, and at various partial pressures of oxygen. In the experiments it was found that, at temperatures above 400° C and at a partial pressure of oxygen in equilibrium with iron and magnetite, fayalite forms readily from finely ground mixtures of silica glass and any of the following: ferrous oxalate, hematite, magnetite, wüstite, or native iron. Silica in excess of that required by the fayalite formula crystallizes as quartz. Small amounts of phases other than fayalite and quartz appeared in the products of these experiments: In charges which were initially ferrous oxalate and silica glass in minnesotaite proportions, a phase with an atomic spacing of 10.2 Å was formed at temperatures between 450° and 600° C; when the charge was held for longer periods under the same conditions, this phase was replaced by another with an atomic spacing of 7.2 Å, which is similar to the basal spacing of greenalite. Still longer treatment of the same material failed to increase the amount of the phase with the 7.2-Å spacing relative to the amount of fayalite and quartz, which made up the bulk of the products of the experiment. Similarly, in experiments starting with native iron and silica glass in either minnesotaite or greenalite proportions, at temperatures above 400° C, a total water pressure of 30,000 psi, and a partial pressure of oxygen in equilibrium with iron and magnetite, small amounts of a phase with an atomic spacing of 13.1 Å form with predominant fayalite and quartz; these small amounts persist after further treatment under the

same conditions, but do not grow at the expense of fayalite and quartz.

In experiments of long duration at temperatures between 300° C and 400° C, mixtures of wüstite and silica glass with an Fe:Si ratio of 3:4 react to give a green isotropic material which has a refractive index near that of minnesotaite, but which gives no X-ray diffraction pattern, even with long exposures on the powder camera. When this material was held for 6 weeks at 350° C, 30,000 psi water pressure, and a partial pressure of oxygen in equilibrium with iron and magnetite, fayalite and silica glass were obtained. Fayalite forms from wüstite and silica glass mixed in greenalite proportions (Fe:Si=3:2) at temperatures as low as 315° C, in spite of the high water pressures. In experiments with other starting materials, such as ferrosilicon alloys, ethyl orthosilicate, and colloidal silica, fayalite was the only identifiable iron silicate formed. Natural fayalite held for 46 days at 250° C, 30,000 psi water pressure, and a partial pressure of oxygen in equilibrium with iron and magnetite showed no sign of decomposition.

Under the conditions of these experiments, it therefore appears either that hydrous silicates of iron are unstable or that fayalite forms metastably and thereafter fails to react with silica and/or water. In nature, fayalite is absent from rocks whose bulk compositions fall in or near the system  $\text{FeO}-\text{Fe}_2\text{O}_3-\text{SiO}_2-\text{H}_2\text{O}$ , except in the highest grades of metamorphism. This makes it highly probable that in the experiments described above, especially in those below about 500° C, fayalite formed metastably, and that equilibrium was not attained. Investigation of this geologically important system under controlled pressures of oxygen therefore remains a challenging problem.



# ISOGRAD PROBLEMS IN METAMORPHOSED IRON-RICH SEDIMENTS

*H. S. Yoder, Jr.*

The iron-rich sediments are sensitive to changes in pressure and temperature; their mineral assemblages are, therefore, very useful as indicators of metamorphic grade. Ferruginous sediments, however, are not common rocks, and their metamorphosed equivalents are even less common on a world-wide basis. For this reason the ferruginous metamorphic rocks have not been studied in the field as intensively as those derived from the dominantly magnesian and aluminous sediments. The principles obtained from field investigations of the magnesium- and aluminum-rich rocks, however, and the physicochemical principles derived from laboratory studies of analogous systems, are directly applicable to the problems in the iron-rich metamorphic rocks.

The metamorphism of two critical types of iron-rich sediments, those containing principally greenalite and those consisting dominantly of the chamosites, will be examined in the light of the established principles. For the most part the rocks containing greenalite can be represented in the system  $\text{FeO-SiO}_2\text{-H}_2\text{O}$ , and those containing chamosite, in  $\text{FeO-Al}_2\text{O}_3\text{-SiO}_2\text{-H}_2\text{O}$ . The members of the two principal mineral groups have a kaolinite-like structure, and it is probable that a complete series of solid solutions exists between the end members greenalite,  $\text{Fe}_6^{+2}\text{Si}_4\text{O}_{10}(\text{OH})_8$ , and  $\text{Fe}_4^{+2}\text{Al}_4\text{Si}_2\text{O}_{10}(\text{OH})_8$ , as yet not named. Substitutions of the type  $\text{Mg} \rightarrow \text{Fe}^{+2}$ ,  $\text{Fe}^{+3} \rightarrow \text{Al}^{+3}$ , and  $\text{Fe}^{+3} \rightarrow \text{Fe}^{+2}\text{H}^{+1}$  are known, but their extent has not been delineated. In addition, more complex substitutions trending from trioctahedral toward dioctahedral character are possible.

The minerals in the  $\text{FeO-SiO}_2\text{-H}_2\text{O}$  system are plotted in figure 61, and the tie lines are those believed to exist at room temperature, for example. Two important concepts may be obtained from a study of this diagram. All the possible phases are

present at the lowest temperatures, yet only those assemblages greenalite + quartz + water (analogous to a sediment) or greenalite + fayalite + water (analogous to a partly serpentinized dunite) are commonly observed. In the sediments, for example, the phases are in equilibrium with water, and, therefore, only those assemblages in which water can occur as a phase are permissible. The remaining assemblages may occur in environments where water does not exist as a phase.

The second concept arises from a consideration of the corner (FeO). The compound wüstite, FeO, which has not been found occurring naturally, is not stable below approximately  $570^\circ \text{C}$ , and its bulk composition is represented by magnetite + iron. In nature most rocks contain magnetite; on the other hand, native iron is exceedingly rare. For the present purposes, therefore, only those assemblages in equilibrium with magnetite will be considered. Oxygen, an important component in the iron-bearing rocks, is itself worthy of a detailed discussion. For the present problems, however, only a few brief remarks are necessary.

In the tetrahedron in the upper left corner of figure 61 are plotted the possible phases in the system  $\text{Fe-Si-O}_2\text{-H}_2$ . The evolution of the Fe-Si-O face of the tetrahedron may be followed from the schematic diagrams in figure 62, which is based on the work of Darken and Gurry (1946). The total pressures in nature far exceed those represented by the top curve in figure 62, which is less than approximately  $10^{-5}$  atm. It is seen in figure 61 (inset) that the bulk compositions of rocks containing the silicates and magnetite must lie in the tetrahedron magnetite-fayalite-quartz-water. Here "water" represents the homogeneous gas phase, the composition of which, although close to  $\text{H}_2\text{O}$ , may be enriched in the component oxygen or the

component hydrogen as well as iron and silica. If the component oxygen is in excess of that of the saturated gas in equilibrium with magnetite and silicates, all the silicates and some or all of the magnetite would be oxidized to an assemblage of magnetite + hematite + quartz + gas or hematite + quartz + gas.

Consider a finely banded rock of alternating layers of magnetite, hematite, and

it is concluded that in these cases oxygen does not diffuse freely from one layer to another. In addition, James (1955) finds as a result of his field studies in Michigan that the oxygen content of a given layer does not appear to change with metamorphism. A hematite + quartz layer persists through all grades of metamorphism. Since none of the silicates with the exception of quartz can coexist with hematite

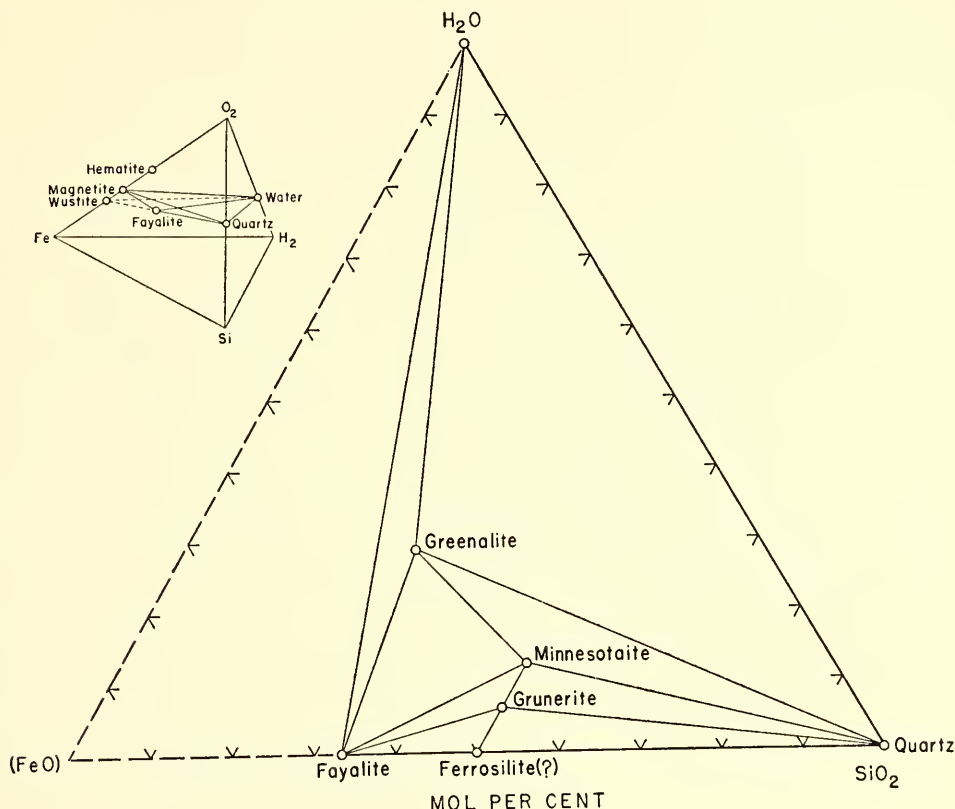


Fig. 61. Projection of the system fayalite-quartz-water-magnetite onto the  $\text{FeO-SiO}_2\text{-H}_2\text{O}$  plane at approximately room temperature. The composition  $\text{FeO}$  is represented by iron + magnetite at this temperature. The inset shows the location of the system in the tetrahedron  $\text{Fe-Si-O}_2\text{-H}_2$ .

a mineral such as greenalite or stilpnomelane which may possess  $\text{FeO/Fe}_2\text{O}_3$  in various ratios. Only the hematite layer could be in equilibrium with a gas phase containing oxygen in excess of the gases in equilibrium with magnetite and the silicates; the others are prohibited from occurring with such a gas. It is believed, therefore, that each layer is in itself essentially a closed system with regard to oxygen. Under equilibrium conditions all the minerals may exist at the same total pressure but with varying oxygen contents of the layers. Since such layering is common,

and a gas containing oxygen in excess of that in equilibrium with magnetite or the silicates, no iron-bearing silicates are formed during metamorphism. In beds initially consisting of magnetite + quartz, the various iron silicates form. The gas plays an important role because it acts as a stabilizer even though the amount of solids far outweighs the amount of gas present.

Considering only those assemblages in equilibrium with magnetite, the sequence of metamorphic changes in beds consisting dominantly of greenalite can be de-



duced. The basis for the deductions is the general principle that the thermal stability of the hydrous minerals increases with decreasing water content. The first triangle in figure 63 is the same as that given in

highest temperature is fayalite+quartz+vapor. At all temperatures, all assemblages are in equilibrium with magnetite. Each of these reactions will be marked by an isograd. If the initial bulk composition of

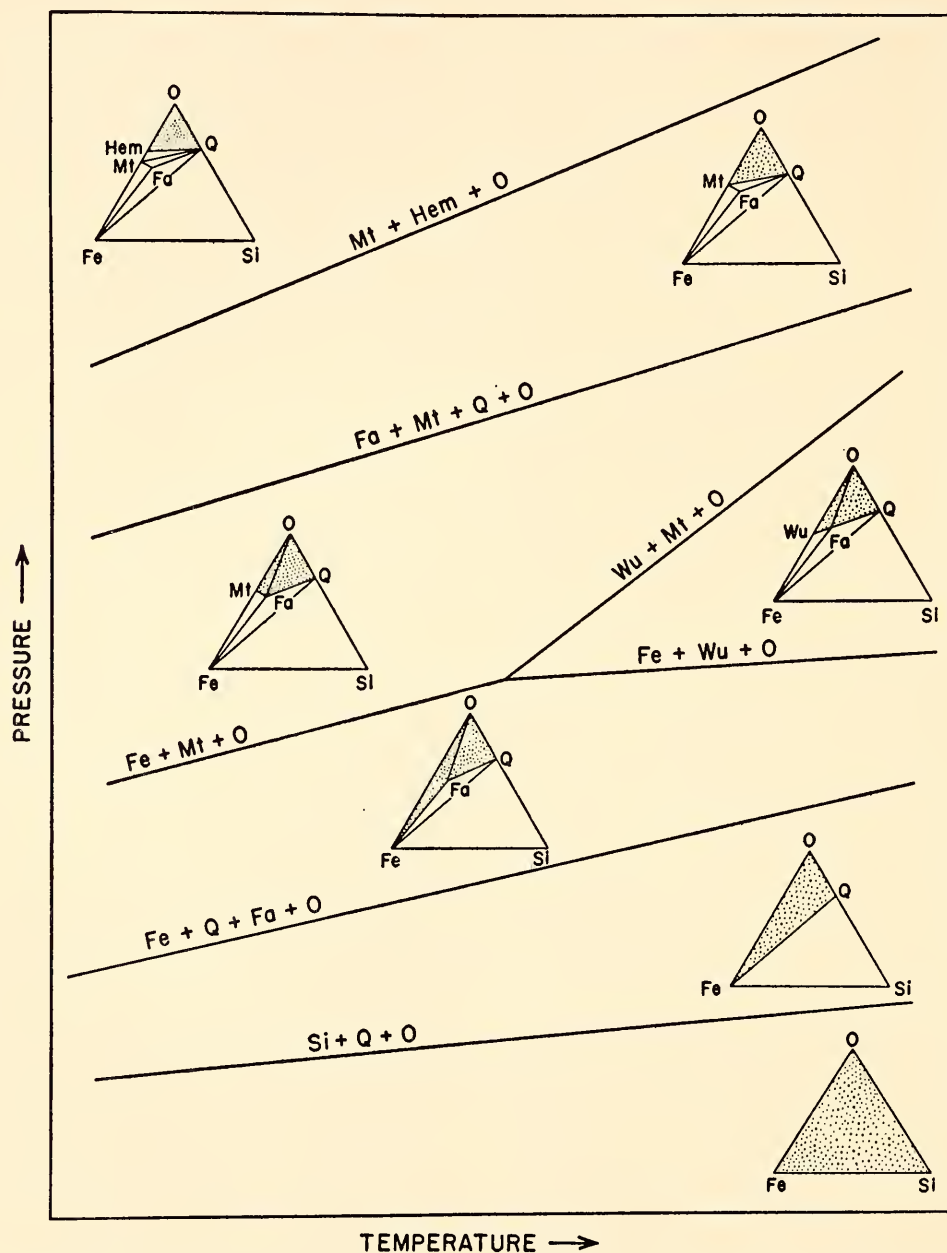


Fig. 62. Schematic representation of the assemblages in the Fe-Si-O system stable at various temperatures and pressures. The curves are based on the work of Darken and Gurry (1946). The stippled areas indicate those bulk compositions for which oxygen exists as a phase.

figure 61. With increasing temperature greenalite reacts with quartz to yield minnesotaite+vapor. Next greenalite decomposes. Minnesotaite reacts with fayalite, and grunerite now appears in the presence of vapor. Minnesotaite breaks down at higher temperatures, and finally grunerite decomposes. The assemblage stable at the

the sediment were in the field greenalite + quartz + water + magnetite, then the index minerals to appear with progressive metamorphism would be minnesotaite, grunerite, and fayalite (or hypersthene). This sequence has been observed in northern Michigan and elsewhere.

The minerals in the  $\text{FeO}-\text{Al}_2\text{O}_3-\text{SiO}_2$ -

$\text{H}_2\text{O}$  system are projected onto the  $\text{FeO}-\text{Al}_2\text{O}_3-\text{SiO}_2$  plane from  $\text{H}_2\text{O}$  in figure 64. All the phases that appeared in figure 61 are projected onto the side line  $\text{FeO}-\text{SiO}_2$ . The chamosites, which are critical to the

quartz is probably detrital. Magnetite may be detrital or may be diagenetic in origin, through the reduction of hematite by organic means, for example. The 2M mica arises from the 1Md mica (illite or glau-

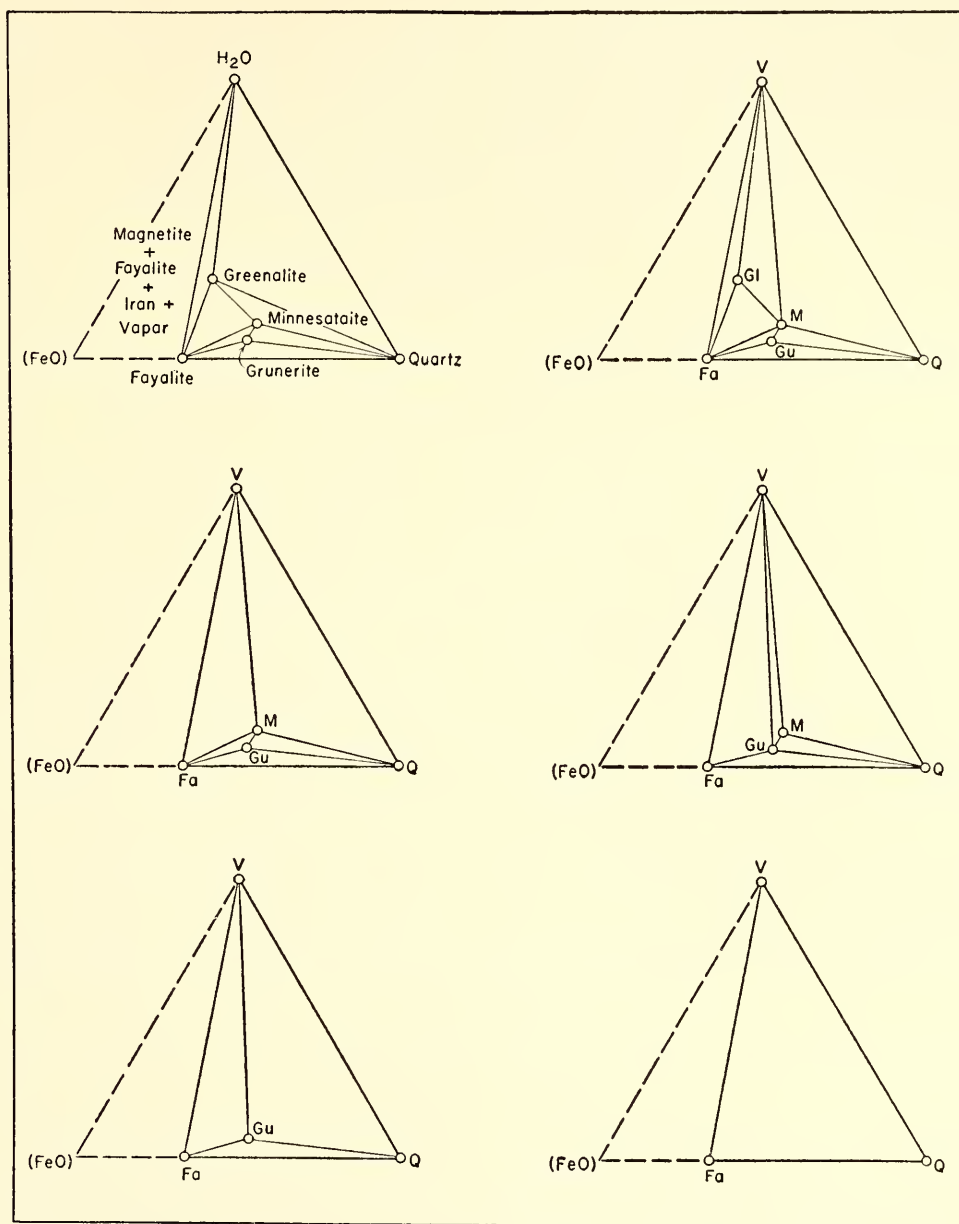


Fig. 63. Assemblages stable at successive elevated temperatures in the system  $(\text{FeO})-\text{Fe}_3\text{O}_4-\text{SiO}_2-\text{H}_2\text{O}$  projected onto the  $(\text{FeO})-\text{SiO}_2-\text{H}_2\text{O}$  plane. The phase " $\text{H}_2\text{O}$ " or " $V$ " represents the homogeneous gas phase.

formation of many of these minerals, are the low-temperature polymorphs of the chlorites that lie along part of the join extending from greenalite to pseudothuringite. At the lowest grade of metamorphism, a schist containing chlorite + 2M-muscovite + quartz + magnetite is usually recognized first. It is of interest to examine the source of even these minerals. The

conite) laid down in the sediment, probably passing through the stages  $1\text{Md} \rightarrow 1\text{M} \rightarrow 2\text{M}$ . It is likely that when the polymorphs of the micas are examined more closely an isograd may be established to mark the  $1\text{M} \rightarrow 2\text{M}$  transition. The iron chlorite, which has a 14-A structure, arises from the polymorphic transition of the 7-A chamosites, marking the chlorite iso-





and, like chloritoid, its presence depends on a high alumina content. There is some doubt as to the exact composition of staurolite. Juurinen's recent formula for staurolite,  $\text{H}_4\text{Fe}_4\text{Al}_{18}\text{Si}_8\text{O}_{48}$ , does not balance in charge. Many analyses closely approach  $\text{FeO} \cdot 2\text{Al}_2\text{O}_3 \cdot 2\text{SiO}_2 \cdot \text{H}_2\text{O}$ , but the water content is usually low and the alumina

gested that, in those rocks in which chloritoid is absent, staurolite forms with quartz at the expense of garnet + kyanite (the reacting pairs may be visualized in fig. 64). The appearance of kyanite at the next higher grade is in some cases dependent on the reverse of this reaction.

Cordierite appears at the highest temperatures in the hornfels of the contact aureoles and is absent or rare in progressive regional metamorphism. It is a breakdown product of many of the hydrous iron silicates.

The first *appearance* of an index mineral as the result of a reaction has been taken as the marker of an isograd. The examples given above, unfortunately, are probably not the only possible ways in which an index mineral may arise. It is clear that the specific reaction marking an isograd must be stated. There is a great need, therefore, for accurately identifying minerals on each side of an isograd. It may be more important to establish the disappearance of a mineral than its appearance, as was emphasized by Bowen (1940) in his discussion of the progressive metamorphism of a siliceous dolomite. Since most of the iron minerals have now been synthesized in the laboratory, quantitative data on the principal reactions will doubtless be forthcoming.

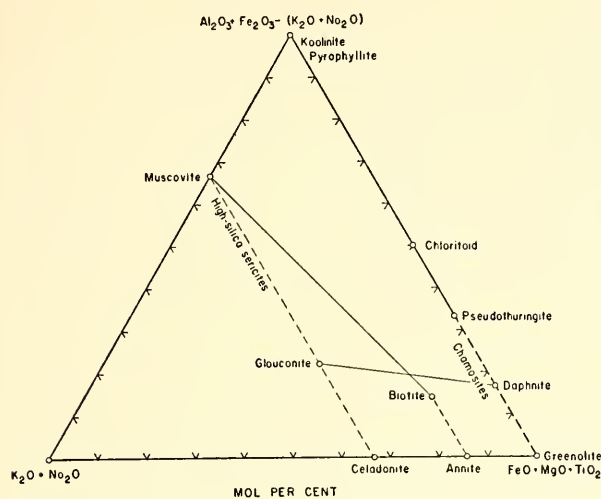


Fig. 65. Projection of the reacting pairs glauconite and daphnite which produce biotite and muscovite. The same products may result from the reaction of a high-silica sericite with daphnite.

content appears to be too high. Possibly  $(\text{Fe,Al})^{+3}$  is replacing  $\text{Fe}^{+2}\text{H}^{+1}$ , as in the chamosites. Staurolite may arise with garnet as the result of the reaction of chloritoid + quartz. Some workers have sug-

## CRYSTALLOGRAPHY

G. Donnay

### SYNTHETIC NEPHELINES

The substitution solid solution  $\text{Na}_{8-x}\text{K}_x\text{Al}_8\text{Si}_8\text{O}_{32}$  is of particular interest because of the two transitions that occur at two definite compositions, namely at  $x \sim 1/4$  and  $x = 2.00$ , first found by J. V. Smith (Year Book 52, pp. 53-56). They were characterized as high-order transitions in a report to the Third International Meeting on Reactivity of Solids (Geophysical Laboratory Paper 1264), which is summarized in the present Year Book. Such transitions differ from the familiar high-order transitions found in metallurgical systems in that they are not associated with changes

in temperature but accompany compositional changes. Only preliminary data had been obtained to locate the transition composition in the neighborhood of  $x = 1/4$ . In view of the interest of such high-order transitions in mineralogical systems, additional work was undertaken (jointly with J. F. Schairer and J. D. H. Donnay) in an effort to check and refine previous results.

Pure sodium nepheline glass was synthesized and crystallized by being held, for various lengths of time, at different temperatures (table 24). Cell dimensions were determined by the method of least squares from X-ray diffraction patterns, obtained



TABLE 24. X-Ray Data for Pure Sodium Nepheline  
(Synthetic samples with different thermal histories)  
 $\text{NaAlSiO}_4$

Thermal History, ° C	<i>a</i> , Å	<i>c</i> , Å	<i>V</i> , Å <sup>3</sup>	<i>c/a</i>
1100°, 6 hr (S)*	9.971†	8.362†	720.0†	0.8386
900°, 20 days (S)	9.984	8.333	719.3	0.8346
900°, 34 days (S)	9.988	8.333	719.9	0.8343
1000°, 6 days (S)	9.986	8.331	719.5	0.8343
1000°, 34 days (S)	9.986	8.328	719.2	0.8340
1050°, 6 days (S)	9.984	8.333	719.3	0.8346
1050°, 34 days (S)	9.991	8.331	720.2	0.8339
1200°, 2 days (S)	9.984	8.328	719.1	0.8341
1200°, 10 days (S)	9.984	8.328	719.1	0.8341
540°, 2000 bars, hydrothermal, 1 week (B) . . .	9.989	8.328	719.6	0.8337

\* (B) Boyd, (S) Schairer.  
† Quenched high-temperature form.

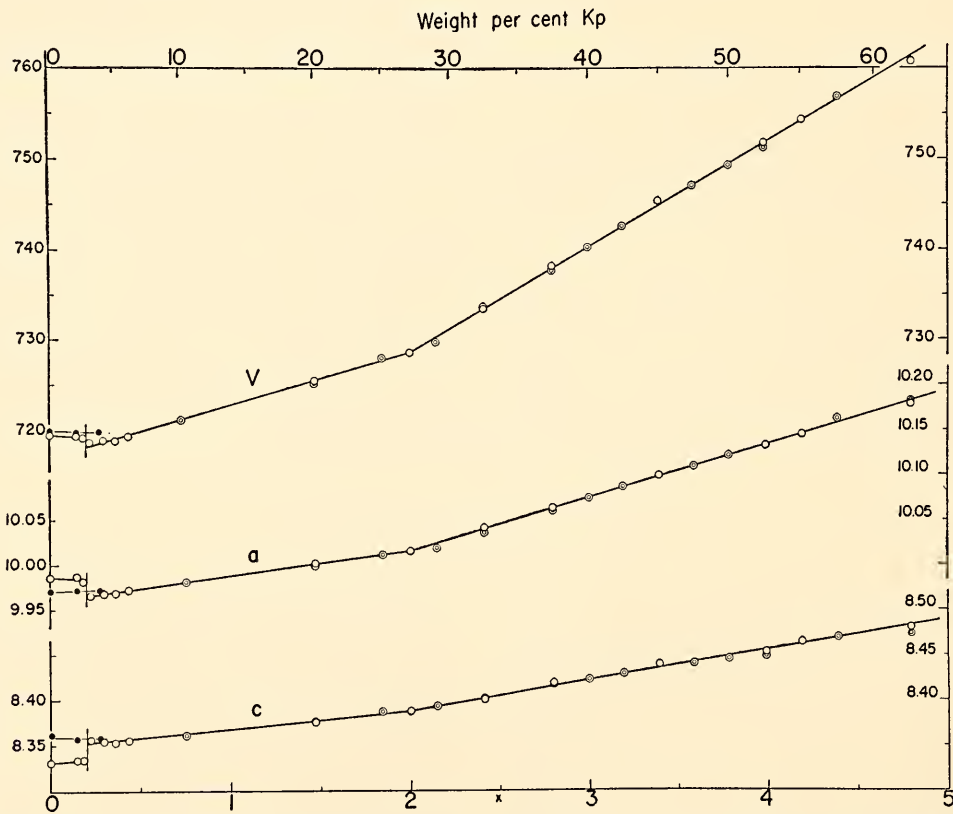


Fig. 66. Change of cell dimensions (*c*, *a* in Å; *V* in Å<sup>3</sup>) in solid solution  $\text{Na}_{8-x}\text{K}_x\text{Al}_8\text{Si}_8\text{O}_{32}$ . The lower abscissa scale gives the number *x* of potassium atoms per cell. The upper scale gives the weight percentage of Kp in the system Ne-Kp, where Ne stands for  $\text{NaAlSiO}_4$  and Kp for  $\text{KAlSiO}_4$ . Black circlets, quenched high-temperature form; white circlets, low-temperature form. For comparison previous data (Geophysical Laboratory Paper 1267) are shown by double rings.

with the Norelco instrument, usually for two samples crystallized at the same temperature but for different lengths of time. The patterns yielded by the samples that were held at 800° C for at least 27 days show one faint nepheline line; they were identified as low-carnegieite patterns. The samples held at 900° C for 20 days did not contain detectable carnegieite; those pre-

cause in one sample a high-temperature form was obtained by quenching; its cell dimensions are  $a=9.971$ ,  $c=8.362$  A,  $V=720.0$  A<sup>3</sup>. Within the limits of accuracy claimed here for the method of cell-dimension determination, we therefore have evidence for the existence of two nepheline forms in the temperature range considered. In contradistinction to albite, which ex-

TABLE 25. X-Ray Data for Low-Potassium Nephelines  
(Synthetic samples with different thermal histories)

Na <sub>8-x</sub> K <sub>x</sub> Al <sub>8</sub> Si <sub>8</sub> O <sub>32</sub>					
Wt. % KAlSiO <sub>4</sub>	<i>x</i>	Thermal History, ° C	<i>a</i> , A	<i>c</i> , A	<i>V</i> , A <sup>3</sup>
2.00.....	0.144	1200°, 8 days (S)*	9.971†	8.356	719.4
		1200°, 5 days (S)	{ 9.989	8.333	719.7 (a)‡
			{ 9.980	8.333	719.2 (b)
2.50.....	0.180	1200°, 8 days (S)	9.973†	8.358	719.9
		1060°, 29 days (S)	9.977	8.341	719.1
		1200°, 6 days (S)	{ 9.970	8.332	719.2
			{ 9.977	8.337	718.6
3.00.....	0.216 <sub>5</sub>	1200°, 72 hr (S)	9.985	8.335	719.7
		1200°, 7 days (S)	9.966	8.356	718.7
3.75.....	0.270	1050°, 12 days (S)	9.971†	8.358	719.6
		1100°, 10 days (S)	9.975†	8.360	720.4
		1200°, 12 days (S)	9.975†	8.358	720.2
4.00.....	0.289	1100°, 10 days (S)	9.969	8.354	719.0
		1200°, 5 days (S)	9.963	8.354	718.3
		1200°, 8 days (S)	9.971	8.355	719.4
5.00.....	0.361	1100°, 7 days (B)	9.968	8.353	718.8
6.00.....	0.434	1200°, 5 days (S)	{ 9.963	8.347	717.2 (a)
			{ 9.971	8.354	718.8 (b)
		1200°, 8 days (S)	9.976	8.361	720.6

\* (B) Bowen, (S) Schairer.  
† Quenched high-temperature form.  
‡ (a) ¼°/minute, (b) ½°/minute.

pared at 1000° and 1050° C for 5 days or at 1200° C for 2 days showed only nepheline. On all the runs but one the cell dimensions (fig. 66) were found to be:  $a=9.986 \pm 0.005$ ,  $c=8.331 \pm 0.004$  A,  $V=719.5$  A<sup>3</sup>. The numerical value of  $a$  ranges from 9.984 to 9.991; that of  $c$ , from 8.328 to 8.333. A sample of pure sodium nepheline crystallized (by F. R. Boyd) hydrothermally at 540° C and 2000 bars for 1 week gave cell dimensions in good agreement with the above values. The above samples prepared at high temperature must have inverted to a low-temperature form on cooling, be-

hibits a unique stable crystalline form for each temperature (MacKenzie, Year Book 55, 1955-1956, p. 188), nepheline shows only two forms. New samples were also prepared by Schairer with compositions in the low-potassium region of the NaAlSiO<sub>4</sub>-KAlSiO<sub>4</sub> solid solution (table 25). Most of the samples transformed to the low-temperature form, for which the following data were obtained by X rays. For 2.50 weight per cent KAlSiO<sub>4</sub>, that is  $x=0.180$ ,  $a$  decreases slightly whereas  $c$  remains constant. For 3.00 weight per cent, or  $x=$



0.2165, large changes are suddenly observed in the samples studied. From this point on, to 27.07 weight per cent, that is  $x=2.00$ , the cell dimensions are found to increase linearly, in agreement with previous results of Smith and Tuttle (Geophysical Laboratory Paper 1267). The compositional transition that takes place near  $x=0.20$  is thus marked by discontinuities in the curves of  $a$  and  $c$ ; the curve of cell volume  $V$ , on the other hand, shows small variation; it consists of two straight-line portions, one with nearly zero slope, the other with positive slope. The compensating effect of the opposite variations in  $a$  and  $c$  accounts for the smallness of the offset between the two line segments of the  $V$  curve in the region in which the transition composition must lie. It is impossible to decide whether the  $V$  curve shows a discontinuity, which would indicate a first-order transition, or a singularity, which would be the sign of a second-order transition. Since we have no evidence in favor of the two-phase region, near  $x=0.20$ , that would be required by a first-order transition, the hypothesis of a second-order transition appears to be the more reasonable one.

The problem of determining the order of a transition on the basis of cell volume is likely to lead to the kind of difficulty encountered here, as it did in the study of high-temperature alkali feldspars (Geophysical Laboratory Paper 1179), in view of the uncertainty of the composition and the limited accuracy (0.05 per cent) with which cell dimensions can be determined by the usual X-ray method.

Some of the synthetic samples of low-potassium nephelines (table 25) gave the high-temperature form on quenching, namely: two samples out of three at  $x=0.144$  (2.00 weight per cent  $\text{KAlSiO}_4$ ) and all three samples at  $x=0.289$  (4.00 weight per cent  $\text{KAlSiO}_4$ ). Their cell dimensions are shown in figure 66, where it may also be noted that the upper limit of potassium content of the high-temperature form is probably  $x=0.3$  or  $0.4$ , where the cell di-

mensions of the high-temperature form merge into those of low-temperature nepheline (beyond the discontinuity of  $x \sim 0.20$ ).

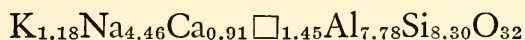
In view of the above results, it was decided to take powder patterns at varying temperature. The Gordon Davis heating sample-holder, recently rebuilt by F. R. Boyd, was used. A sufficiently large amount of synthetic pure sodium nepheline could be gathered from the samples available. The chief difficulty came from the lack of standard material with interplanar spacings calibrated at different temperatures. Inasmuch as the purpose of the experiment was to ascertain the existence of a transition, already suggested by previous results, rather than to make absolute measurements of spacings or to determine the transition point with accuracy, uncorrected  $2\theta$  readings would be satisfactory.

The range of  $2\theta$  extending from  $26^\circ$  to  $30^\circ$  C was covered twice, that is, with increasing and decreasing  $2\theta$ , for each of the following temperatures:  $240^\circ$ ,  $410^\circ$ ,  $610^\circ$ ,  $810^\circ$ ,  $1050^\circ$ , and  $1150^\circ$  C. The average value of  $2\theta$  was plotted (fig. 67) for two spacings:  $20\bar{2}2$  and  $21\bar{3}0$ . The curve of  $2\theta(20\bar{2}2)$  shows a break in the neighborhood of  $810^\circ$  C; it is difficult to confirm the break by means of the curve of  $2\theta(21\bar{3}0)$ , which by itself can be regarded as a straight line within the limits of error. We note that the temperature scale is only roughly calibrated, so that the values given here may be in error by about  $\pm 50^\circ$  C, but this fact does not invalidate the conclusion that a transition exists. Smith and Tuttle (Geophysical Laboratory Paper 1267) place the transition in the neighborhood of  $900^\circ$  C.

#### A SODIUM NEPHELINE IN NATURE

In the course of a study of nepheline solid solutions (jointly with J. F. Schairer and J. D. H. Donnay), we had the opportunity to gather from the literature a large number of chemical analyses of nephelines, together with corresponding cell dimen-

sions determined by X rays. Among them one sample stands out as abnormal, in that its cell dimensions are quite different from those of all the others. It is a sample of nepheline from Monte Somma (British Museum No. 51495), described by Bannister (1931). The chemical analysis can be recast in the following formula



on the basis of the 32 oxygen atoms contained in the cell. The cell dimensions,

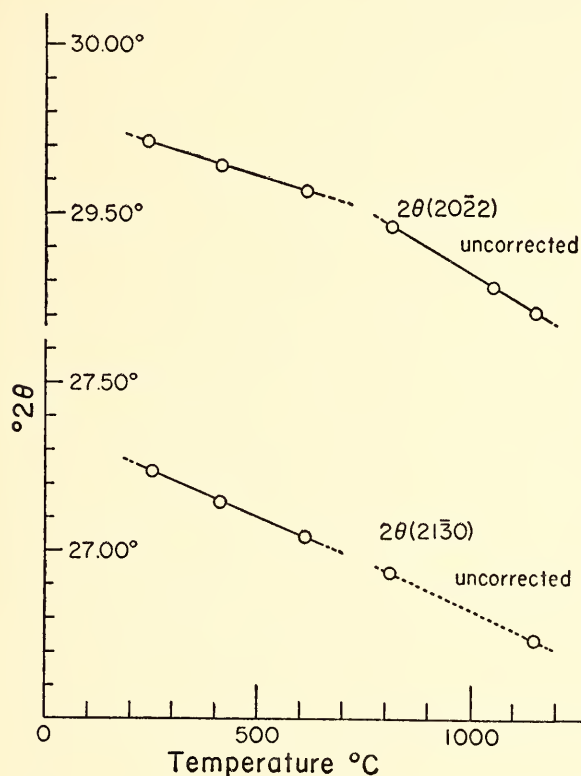


Fig. 67. Uncorrected  $2\theta$  angles obtained with  $\text{CuK}\alpha$  radiation for spacings  $20\bar{2}2$  and  $21\bar{3}0$  of synthetic pure sodium nepheline at varying temperatures ( $^{\circ}\text{C}$ ,  $\pm 50^{\circ}$ ). The transition between low-temperature and high-temperature forms is marked by a break in the curve of  $2\theta(20\bar{2}2)$ .

determined by Bannister by the oscillating crystal method, are:  $a=9.96$ ,  $c=8.33$  (Å from kX), which lead to a calculated density of  $2.645\text{ g/cm}^3$ . The reported observed density,  $2.576\text{ g/cm}^3$ , is unusually low. No other natural nepheline has so low a value for  $c$ .

Dr. M. Hey (Mineral Department of the British Museum) kindly sent us part of the original sample, consisting of fragments, among which could be found a

small euhedral crystal suitable for X-ray work. Its cell dimensions were determined on the precession camera:  $a'=9.988$ ,  $c'=8.328$  Å, both  $\pm 0.3$  per cent,  $c/a=0.8338$ , in agreement with Bannister's results.

Another portion of the sample was used for powder work on the Norelco diffractometer; it gave:  $a''=9.985$ ,  $c''=8.372$  Å, both  $\pm 0.05$  per cent,  $c/a=0.8385$ . Even though  $a'=a''$  within the limits of error, it can be concluded that the sample contains two phases, for the difference in  $c$  exceeds the uncertainty of the measurements. The phase detected as a single crystal must exist in the sample in very small quantity, as its effect on the powder pattern is not noticeable. Its cell dimensions are those obtained for samples of sodium nepheline synthesized by Schairer (see above), with less than 0.20 potassium atom per cell ( $x<0.20$ ).

It is interesting to note that Cesàro (1920) made a careful goniometric study of small perfect crystals from Monte Somma, in which he was able to measure the angle  $(10\bar{1}0):(10\bar{1}1)$  to 2 or 3 minutes of arc. The lowest  $c/a$  ratio he records is 0.8358. This value is lower than the  $c/a$  ratio 0.8381, which is the smallest value found by X rays in the low-potassium region ( $0.20<x<2.00$ ). Cesàro too seems to have encountered a specimen of sodium nepheline in his Monte Somma material.

In conclusion, the chemical formula given above cannot represent any one of the specimens used for single-crystal work, although it may approximate the composition of the powder sample.

Bannister had already shown, by means of optical measurements, that the chemical composition of the material occasionally changes from grain to grain in one hand specimen. Such variability in composition has, of course, long been recognized in mineralogy; it must be reckoned with in any attempt to determine chemical composition from cell dimensions. It can only be hoped that careful sampling and grinding of the sample will yield a representative powder, whose X-ray pattern will



show broadened peaks, each peak being smeared over an angular range that corresponds to the range in composition.

#### SOLID SOLUTION

Under this heading last year's report (Year Book 55, p. 205) gave an account of the variation of cell dimensions of synthetic nephelines with different kinds of solid solutions. One of the conclusions was that neither omission solid solution,  $\text{Na}_{8-y}\square_y\text{Al}_{8-y}\text{Si}_{8+y}\text{O}_{32}$ , nor substitution-omission solid solution,  $\text{Na}_{8-2z}\text{Ca}_z\square_z\text{Al}_{8-\text{Si}_8}\text{O}_{32}$ , changes the cell dimensions in either  $a$  or  $c$ . Further work has shown that this conclusion must be amended.

When the work was repeated on newly prepared samples (see Synthetic Nephelines, above), the  $a$  value had to be corrected from 9.971 to 9.986 Å, and the  $c$  value from 8.362 to 8.331 Å; the cell volume, however, changed only slightly, from 720.0 to 719.5 Å<sup>3</sup>. The numerical values given in last year's report are indeed the cell dimensions of crystals of the high-temperature form obtained by quenching. The new data pertain to the low-temperature form, into which the samples inverted on cooling. By a curious coincidence these same cell dimensions are found on crystals which represent the limit of solid solution by substitution-omission, and they also approximate those of the low-potassium substitution solid solution near  $x=0.3$ .

#### PHOSPHATES

The question of stereoisomerism of tetrametaphosphate has been raised by Drs. R. J. Gross and J. W. Gryder (Johns Hopkins University). The existence of two stereoisomers of  $\text{P}_4\text{O}_{12}$ , namely a ring form and a boat form, was postulated by them on the basis of chemical evidence. The two forms can be isolated only in the solid state; by single-crystal work they were proved to be distinct crystalline species. Preliminary results were reported jointly to the American Chemical Society.

Work on the crystallography of alkali phosphates has been continued with Drs.

J. W. Gryder and Helen M. Ondik. A compound reported in the literature as  $\text{Na}_2\text{H}_2\text{P}_4\text{O}_{12}$  was found to be surprisingly insoluble in water for a metaphosphate, and its fibrous habit was intriguing (Griffith, 1956). The compound was synthesized by Griffith's procedure, and its identity was checked by comparing its powder pattern with that of material kindly furnished by Dr. Griffith. The transparent, colorless product is found to consist of two distinct crystalline forms intimately intergrown.

Form I comprises approximately 10 per cent by weight of the sample, as estimated from the relative intensities of powder lines. It consists of crystals of thick tabular habit and average dimensions  $0.3 \times 0.05 \times 0.02$  mm. They are monoclinic, elongated [010], with cell dimensions  $a=30.7$ ,  $b=6.77$ ,  $c=7.12$  Å, all  $\pm 0.5$  per cent,  $\beta=94^\circ 6' \pm 10'$ ,  $V=1476$  Å<sup>3</sup>. A pronounced pseudo-repeat  $a'=a/2$  is evident. Because it proved impossible to separate a sufficient amount of phase I for accurate density determination, we can report only that its density is 2.62 g/cm<sup>3</sup> or greater as determined by the flotation method using a mixture of bromoform and toluene as the inert liquid. For the same reason a chemical analysis of this phase was not possible. The space group is uniquely determined as  $P2_1/a$  by the systematic absences  $h0l$  with  $h$  odd and  $0k0$  with  $k$  odd. Only the forms {100} and {001} are observed, the larger faces being those of {100}. As cleavage is extremely fibrous along  $b$ , good single crystals are difficult to obtain. No further work on this form is contemplated.

Form II, the bulk of the material, consists of thin to thick rectangular plates which grow up to 5 mm in length. Only cleavage fragments can be removed from the matrix of the melt, and therefore morphology gives no clue concerning the point-group symmetry. Cell dimensions were determined on precession films taken with  $\text{MoK}\alpha$  radiation:  $a=18.74$ ,  $b=14.79$ ,  $c=7.03$  Å, all  $\pm 0.3$  per cent,  $\beta=90^\circ 0' \pm 5'$ ,  $V=1948$  Å<sup>3</sup>. Parallel to {100} the cleavage is very easy and very good; parallel to

{010} it is easy and fairly good; and parallel to {110} it is fairly easy and good. The four cleavage directions result in platy to fibrous fragments.

The cell is monoclinic but markedly pseudo-orthorhombic. Although the zero-level net (010)\* shows symmetry  $2_2$ , parallel upper levels show that  $a^*$  and  $c^*$  are not symmetry directions. The pseudo-orthorhombic character is emphasized by the optical orientation—the principal axes of the index ellipsoid lie along the crystallographic axes within experimental error. The optical character is biaxial positive with the plane of the optic axes parallel to (100) and the acute bisectrix along [001]. The indices are  $n_a = 1.485 \pm 0.005$ ,  $n_\beta = 1.510 \pm 0.005$ ,  $n_\gamma = 1.545 \pm 0.001$  (determined by J. D. H. Donnay). Since the systematic absences are  $hkl$  with  $h+l$  odd and  $h0l$  with  $h$  and  $l$  both odd, the space group is  $B2/a$  or  $Ba$ . The  $B$ -centered lattice is used to bring out the pronounced pseudo-symmetry with pseudo space group  $Bmam$ ,  $Bma2$ , or  $B2am$ . The test for pyroelectricity with liquid nitrogen gave negative results.

Because form II is present in much larger amounts than form I, its density could be determined more accurately. The lowest density obtained by the pycnometric method using toluene as the inert liquid is  $2.34 \pm 0.02$  g/cm<sup>3</sup>. The flotation method using a mixture of bromoform and toluene indicates that the density of fragments of the crystal intergrowth varies from 2.34 to 2.62 g/cm<sup>3</sup>.

Using 2.34 g/cm<sup>3</sup> as the density and Griffith's formula, we calculate  $7.54 \pm 0.08$  formula units per cell. The space groups  $B2/a$  and  $Ba$  permit only an even number of  $\text{Na}_2\text{H}_2\text{P}_4\text{O}_{12}$  units per cell; consequently the observed density must be compared to the calculated densities of 1.86 for 6 and 2.48 for 8 units per cell. These values lie well outside the limits of experimental error. If one molecule of water is subtracted from the empirical formula, the result is  $\text{Na}_2\text{P}_4\text{O}_{11}$ . For 8 such formula units per cell the calculated density is 2.36 g/cm<sup>3</sup>,

within the limits of error of the experimental value. Knowing that the phosphorus atom surrounds itself tetrahedrally by oxygen atoms, we conclude from the formula that some of the  $\text{PO}_4$  tetrahedra must share more than two corners. Following van Wazer's terminology (1955), the material is therefore an ultraphosphate. It is the first crystalline ultraphosphate on record.

#### DISORDER IN CRYSTALS

Present-day interest in crystal structures has shifted from the regularity to the imperfections of the interatomic arrangement. Considerable attention has already been devoted to the study of mistakes that occur during crystal growth or from the very mechanism of growth, such as screw dislocations. Order-disorder studies in recent years have had a profound impact on mineralogy, particularly on our knowledge of layer minerals and of feldspars. A study of disorder by means of optical diffraction has been initiated in this laboratory (Chayes). In view of the growing importance of this field, new types of crystal disorder are of special interest.

Such a new type has been encountered in sodium ultraphosphate form II (with Gryder and Ondik; see Phosphates, above). The experimental evidence is as follows. On the precession patterns containing reflections  $hk0$ ,  $hk1$ , and  $hk3$ , all reflections with  $l$  even are sharp, while those with  $l$  odd appear as diffuse circles. The  $c$ -axis rotation pattern consists of odd layer lines which are diffuse streaks and of even layer lines which contain sharp spots, the width of the two types of layer lines being the same. It follows that the reflections with  $l$  odd are diffuse circular disks oriented normal to the  $c^*$  axis. This conclusion is confirmed by Weissenberg photographs. The intensity distribution within the streaky layer lines varies from crystal to crystal. The radius of the disk was measured for one of the specimens and was found to be  $0.021 \pm 0.001$  Å<sup>-1</sup>.

These observations indicate that the crys-



tal structure is disordered by random displacements of structural elements through a distance of  $c/2$  in the  $z$  direction. The bonds parallel to the  $c$  direction must be very much stronger, indeed of a different order of magnitude, than the bonds parallel to (001). Such requirements are met by a chain structure with chain axes parallel to  $c$ .

Additional evidence leads to the hypothesis that rings of four phosphate tetrahedra are linked into chains by the sharing of an oxygen atom between consecutive rings. The cell height being equal to the height of one such ring, the disordered crystal consists of chains displaced by half a link with respect to one another. An expression relating the observed diffuse intensities to the probability of chain displacement has been derived; the theoretical treatment is similar to that given by Wilson for layer displacements.

#### POLYMORPHISM VERSUS ISOMERISM

When a chemical compound exists in more than one crystalline modification, it is usually a case of polymorphism, in which each of the polymorphic forms is stable in a definite region of the  $P$ - $T$  diagram. If the crystal structure of the compound is of the molecular type, polymorphic forms differ in the way identical molecules pack in the crystal, whereas isomeric forms differ in the molecular configuration itself and separate on crystallizing from a solution in which they co-exist in equilibrium. To discriminate between polymorphism and isomerism, organic chemistry usually must come to the rescue of crystallography unless the crystal structure is first completely determined. The working crystallographer, however, may have occasion to suspect the existence of isomerism in the crystals he studies, and can draw the chemist's attention to the problem. One such opportunity came to us this year.

Dr. F. W. Barnes, of the Johns Hopkins Hospital, had given us hollow crystals of 5,5-diethylbarbituric acid (barbital, vero-

nal), grown at low temperature in his laboratory. These crystals turned out to be rhombohedral, thus different from the monoclinic pseudo-orthorhombic barbital described in the chemical and crystallographic compendia. Mr. William Seip then referred us to a paper by Fischer and Kofler, that appeared in the *Archiv der Pharmazie* in 1932, in which three forms of barbital, described as polymorphic forms, had been studied optically with the polarizing microscope. A crystallographic re-examination of these forms was undertaken (jointly with J. D. H. Donnay).

New data were obtained on the rhombohedral form. It occurs in elongated crystals composed of trigonal prisms:  $11\bar{2}0$ ,  $21\bar{1}0$ ,  $30\bar{3}0$ ,  $03\bar{3}0$ ,  $41\bar{5}0$ , and  $\bar{1}540$ , in order of decreasing size. Several easy cleavages are parallel to the  $c$  axis. Cleavage fragments were used for X-ray work. Cell dimensions are:  $a=26.97\pm0.09$ ,  $c=6.85\pm0.02$  Å; 18 molecules per cell give a calculated density of  $1.276$  g/cm<sup>3</sup>, as compared with the measured value of  $1.26$  (by M. Crute). The space group determined by X rays is  $R\bar{3}$  or  $R3$ ; a pyroelectric test in liquid nitrogen was negative, but  $R3$  is the more probable in view of the morphological development. A powder pattern was taken and indexed. The indices of refraction determined by Fischer and Kofler were confirmed to 0.001.

Crystal data on the monoclinic form of barbital go back to Hertel (1930, 1935). In the pseudo-orthorhombic description (1930), Hertel had given an incorrect space group,  $Cmcm$  instead of  $Ccmm$ , for  $a=7.11$ ,  $b=14.4$ ,  $c=9.7$  kX. The morphological development of the crystals was found to be incompatible with the space group on record; re-examination by X rays gave the correct space group. The monoclinic description (1935) with space group  $C2/c$  or  $Cc$  and interchange of  $a$  and  $b$  was confirmed by indexing a powder pattern. The optical data of Fischer and Kofler (1932) were confirmed; the indices agree within  $\pm0.003$ . These two authors

observed the  $c$  axis to be polar; the probable space group is accordingly  $Cc$ .

Preliminary data on the triclinic form confirm the values of the indices of refraction measured by Fischer and Kofler on twinned crystal plates. More work is needed before the triclinic character can be confirmed and cell dimensions reported.

The following evidence indicates that the three forms of barbitol are not polymorphs, but isomers. On melting either the monoclinic or the rhombohedral form, and letting it recrystallize from the melt, we always retrieve the original form. Whereas Fischer and Kofler report the rhombohedral form as the form stable at high temperature, the rhombohedral crystals we studied were obtained at low temperature and were accompanied by only very small amounts of the other two forms. Fischer and Kofler report that all three forms usually occur together in their preparations; occasionally only two forms were found by us.

#### DIGENITE

The work on digenite was continued. In view of the impossibility of producing true single crystals, it was decided to attempt a determination of the crystal structure from the data obtained from twins. It was necessary to analyze the data with a view to finding which reflections were contributed by the several crystals of the twin to the cubically indexed diffraction spectra on the X-ray photographs.

A tentative crystal structure has been arrived at, on the basis of space group  $R\bar{3}m$ . All atoms lie along the threefold axis of the rhombohedral cell described in last year's report (Year Book 55, p. 204). The sulfur atoms are placed at  $x=0, \pm\frac{1}{5}, \pm\frac{2}{5}$ , and lead to an S-S distance of 3.92 Å. The sulfur sites correspond to the face centers of the small cube, which was the cell reported by Rahlfs (1936). One copper atom is located at  $x=\frac{1}{2}$ , and is octahedrally co-ordinated to sulfur atoms. The other copper atoms are found at  $x=\pm\sim 0.060$  in tetrahedral co-ordination,

but displaced from the center toward one face of the sulfur tetrahedron; at  $x=\pm 0.133$ , in triangular co-ordination; at  $x=\pm 0.250$  and  $\pm 0.350$ , in regular tetrahedral co-ordination. The Cu-S distance ranges from 2.26 Å for the triangular co-ordination to 2.77 Å for the octahedral one. This structure leads to satisfactory agreement between calculated and observed intensities for the superstructure reflections, which are the reflections that appear on a powder pattern. For the weak reflections, observed only on "single-crystal" patterns, the agreement still leaves much to be desired.

Powder patterns were taken at high temperature at the National Bureau of Standards, on the heating camera designed by Mr. F. A. Mauer, who kindly agreed to help us with this task. The sample was heated in a helium atmosphere to prevent oxidation. The temperature was slowly raised to  $\sim 500^\circ$  C. Between  $60^\circ$  and  $65^\circ$  the weak reflections disappear, indicating the existence of a transition above which the small cubic pseudocell becomes the true cell.

The electrical conductivity of digenite was tested by Dr. A. Franklin, at the National Bureau of Standards. He reports that digenite is an unusually good conductor, even at room temperature; there is no sharp change in conductivity at the transition temperature.

#### CRYSTALLOCHEMICAL ANALYSIS

Identification of small amounts of crystalline material is a problem that is always with us. A number of methods are well established; for instance, determination may be made by means of the polarizing microscope, by the powder technique of X-ray diffraction, by microchemical tests, by spectroscopic analysis. When single crystals are available, identification may be based on the determination of the cell dimensions by X rays, a method that has become possible only recently, after the necessary determinative tables of crystal data were published. It was thus a chal-



lenge to devise a practical determinative procedure that could be applied by scientists not specially trained in X-ray crystallography. Such a procedure has been worked out for each of the crystal systems, and for all kinds of crystals, euhedral, subhedral, or anhedral. It requires only a precession camera and a two-circle goni-

ometer. It can be applied in a routine fashion, with a minimum of interpretation, although some judgment remains indispensable.

The method has been written up as a chapter for the coming third edition of *Physical Methods of Organic Chemistry*, edited by A. Weissberger.

## APPLICATION OF THE "MOREY-SCHREINEMAKERS' THEOREM OF COINCIDENCE"

G. W. Morey

A large part of the experimental work of the Geophysical Laboratory has been the determination of the melting points and other phase-equilibrium relations of the oxides and their mixtures and compounds which make up the earth's crust. This work has been guided by the theoretical precepts developed by the great American physicist and mathematician, J. Willard Gibbs. The greater part of our studies have dealt with nonvolatile oxides, in which the only experimental variables needing consideration are the temperature and the compositions of matter under consideration. There is, however, increasing interest in systems in which volatile substances such as water and carbon dioxide are introduced as components. This increasing interest is manifest not only in the work of this Laboratory but also in other laboratories interested in experimental geochemistry, and to a rapidly increasing extent in the chemical industries, which see the possibilities of radically new methods of manufacture resulting from high-pressure reactions. Such reactions require simultaneous consideration not only of the composition variables but also of pressure and temperature, and involve complications resulting from critical phenomena and significant solubility of solids in gases. The theoretical consideration of such systems is little known and is not ordinarily treated in textbooks.

The fundamental considerations developed by Gibbs are competent guides to the most complicated of these systems, but their detailed application leads to phase-

equilibrium relations difficult to understand and expound because of their complexity. One aspect of such relations, namely the course of the pressure-temperature curves of univariant equilibria in which such  $P$ - $T$  curves follow each other around an invariant point, can be developed and applied in a comparatively simple manner. The treatment of this problem is based on a theorem deduced rigorously by Morey and Williamson, and from general considerations by Schreinemakers, and called by Prigogine the "Morey-Schreinemakers' Theorem of Coincidence." This is a powerful tool, applicable to all types of  $P$ - $T$  curves, and simple to apply, but since it is not included in textbooks it is not generally known. By application of this theorem it is possible not only to determine the sequence of  $P$ - $T$  curves around an invariant point in a system of many components, but also to fix the phase assemblages that can have stable coexistence in the divariant regions between the  $P$ - $T$  curves, even though the points in such regions are projections on the  $P$ - $T$  plane of an  $n$ -dimensional hyperprism. A paper recently published gives a detailed exposition of how this theorem may be applied to the various types of invariant points in a ternary system, and of the various types of  $P$ - $T$  curves proceeding from these invariant points, using as an example the ternary system water- $\text{Na}_2\text{O} \cdot \text{Al}_2\text{O}_3 \cdot 2\text{SiO}_2$  (nepheline)- $\text{Na}_2\text{O} \cdot \text{Al}_2\text{O}_3 \cdot 6\text{SiO}_2$  (albite), in which the compounds  $\text{Na}_2\text{O} \cdot \text{Al}_2\text{O}_3 \cdot 4\text{SiO}_2 \cdot 2\text{H}_2\text{O}$  (analcite) and  $\text{Na}_2\text{O} \cdot \text{Al}_2\text{O}_3 \cdot 4\text{SiO}_2$  (jadeite) are formed.

## MISCELLANEOUS ADMINISTRATION

## PETROLOGISTS' CLUB

The Petrologists' Club met at the Laboratory on six occasions this year. After rather rapid growth in the previous few years, the membership has leveled off at about 150 active participants.

The following papers were presented:

"Gravity and continental structure," by H. E. Tatel (Department of Terrestrial Magnetism).

"Equilibrium texture in rocks," by J. B. Thompson, Jr. (Harvard University).

"Origin of spilites," by G. D. Nicholls (University of Manchester).

"The 1955 eruption of Kilauea," by G. A. MacDonald (U. S. Geological Survey).

"Oxidation and reduction in metamorphism," by H. P. Eugster, J. R. Smith, and W. G. Ernst (Geophysical Laboratory), and H. James (U. S. Geological Survey).

"Origin of lamprophyres associated with granitic plutons," by C. A. Hopson (Johns Hopkins University).

## SEMINARS

The Laboratory continued its weekly series of seminars, in which papers concerned primarily with work in progress were presented largely by staff members. Several talks were given by guest speakers from outside the Laboratory, including:

"The origin of lamprophyres," by C. A. Hopson (Johns Hopkins University).

"An attempt to limit the possible composition of the ore-forming fluid," P. B. Barton, Jr. (U. S. Geological Survey).

"Recent developments in X-ray fluorescence," I. Adler (U. S. Geological Survey).

"Phase relations in the system gold-silver-tellurium," N. Markham (University of Adelaide, South Australia).

"Systems involving ferrous and ferric oxide," A. Muan (Pennsylvania State University).

"Recent studies on silica," R. Roy (Pennsylvania State University).

## SYMPOSIUM ON HIGH PRESSURES

On June 12, 1957, a one-day symposium on high pressures was held at the Geophysical Laboratory. The morning session

was devoted to formal talks by Professor H. Tracy Hall, Director of Research, Brigham Young University, on "Chemistry at high pressures and high temperatures," and by Professor Harry G. Drickamer, Head, Chemical Engineering Department, University of Illinois, on "The effect of high pressures on optical properties of materials." After a buffet luncheon, the first session of the afternoon was devoted to general discussion of phase changes induced by pressure and their relation to geophysical problems, with Professor Birch, of Harvard, contributing notably. The final portion of the day was devoted to apparatus design, with discussion led by Professor Hall.

The group attending the symposium included: L. H. Adams (National Bureau of Standards), L. T. Aldrich (Department of Terrestrial Magnetism), Charles W. Beckett (National Bureau of Standards), Francis Birch (Harvard University), E. H. Carnevale (Naval Ordnance Laboratory), Harry G. Drickamer (University of Illinois), Abraham Friedman (Atomic Energy Commission), Irving Friedman (U. S. Geological Survey), H. R. Gault (Lehigh University), John W. Graham (Department of Terrestrial Magnetism), H. J. Hadow (United Kingdom Scientific Mission), H. Tracy Hall (Brigham Young University), Joseph Hilsenrath (National Bureau of Standards), Francis T. McClure (Applied Physics Laboratory), Donald Newhall (Harwood Engineering Company), Thomas B. Nolan (U. S. Geological Survey), Donna Price (Naval Ordnance Laboratory), Sidney G. Reed, Jr. (Office of Naval Research), A. E. Ringwood (Harvard University), Eugene Robertson (U. S. Geological Survey), William W. Rubey (U. S. Geological Survey), Paul A. Scherer (Carnegie Institution of Washington), H. E. Tatel (Department of Terrestrial Magnetism), Dudley Taylor (Naval Ordnance Laboratory), M. A. Tuve (Department of Terrestrial Magnetism), Alvin van Valkenburg, Jr. (Na-



tional Bureau of Standards), Charles E. Weir (National Bureau of Standards), George W. Wetherill (Department of Terrestrial Magnetism), and Samuel Zerfoss (National Bureau of Standards).

In addition to the above, the following staff members and guest investigators of the Geophysical Laboratory were in attendance: P. H. Abelson, R. G. Arnold, H. L. Barnes, P. B. Barton, Jr. (U. S. Geological Survey), F. R. Boyd, Jr., S. P. Clark, Jr., G. L. Davis, J. L. England, W. G. Ernst, H. P. Eugster, J. W. Greig, G. Kullerud, E. H. Roseboom, J. F. Schairer, J. R. Smith, D. B. Stewart (U. S. Geological Survey), D. R. Wones, H. S. Yoder, Jr., and E. G. Zies.

#### LECTURES

During the report year staff members were invited to present lectures as follows:

P. H. Abelson lectured at the National Academy of Sciences; the Annual Meeting of the Trustees of the Carnegie Institution of Washington; the New York Academy of Sciences-A.A.A.S. Symposium on Modern Ideas on Spontaneous Generation; a graduate seminar at Catholic University; the Pittsburgh Section of the American Chemical Society; a Colloquium in Earth Sciences at the Massachusetts Institute of Technology; the National Biophysical Conference; the U. S. Weather Bureau; the Journal Club of the Department of Geology, Johns Hopkins University; a Symposium on Isotope Separation sponsored by the Netherlands Physical Society and the International Union of Pure and Applied Physics at Amsterdam; and the National Bureau of Standards.

F. R. Boyd, Jr., lectured at the National Academy of Sciences; the Washington Junior Academy of Sciences; and the Physics Department, Howard University.

G. Donnay addressed the Point-Group Seminar, Physics Department, Polytechnic Institute of Brooklyn. Together with G. Kullerud and J. D. H. Donnay (of Johns Hopkins University) she presented a sym-

posium to the Washington Crystal Colloquium.

H. P. Eugster served as Lecturer in the Department of Geology, Johns Hopkins University, each Friday during the academic year 1956-1957. He also lectured at the fall meeting of the National Academy of Sciences.

J. W. Greig served as Visiting Research Associate at the College of Mineral Industries, Pennsylvania State University, from November 1956 through March 1957. During this period he gave a series of lectures on various aspects of phase equilibria in ternary systems, which were attended by staff members and graduate students of five departments: Mineralogy and Petrology, Geophysics and Geochemistry, Geology, Metallurgy, and Ceramic Technology.

G. W. Morey gave a series of three lectures before the Inorganic Chemical Division of Monsanto Chemical Company at Dayton, Ohio.

J. F. Schairer delivered the Presidential Address, "The crystallization of rock-forming minerals from magmas and the nature of the residual liquid," at the annual meeting of the Geological Society of Washington.

G. R. Tilton lectured at the Gordon Research Conference; Georgetown University; a Conference on Nuclear Processes in Geologic Settings at Boston; and a Tektite Conference sponsored by the Division of Earth Sciences, National Research Council.

H. S. Yoder, Jr., lectured at the Department of Geology, Columbia University; a Basalt Conference sponsored by the Division of Earth Sciences, National Research Council; and the Institute on Lake Superior Geology, Michigan State University. He also gave a series of two lectures each, at the Department of Geology, University of Wisconsin; the Department of Geology, University of Chicago; and the Department of Geology, University of Illinois.

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The "Summary of Published Work" below briefly describes the papers published

in scientific journals during the report year. In addition, the following papers are now prepared for publication: H. L. Barnes and G. Kullerud, "Relations between composition of ore minerals and ore solutions"; F. R. Boyd, Jr., "Geology of the Yellowstone rhyolite plateau"; F. Chayes and W. S. MacKenzie, "Experimental error in determining certain peak locations and distances between peaks in X-ray (powder) diffractometer patterns"; G. Donnay, J. D. H. Donnay, and G. Kullerud, "Crystal and twin structure of digenite,  $\text{Cu}_9\text{S}_5$ "; J. W. Gryder, H. Ondik, and G. Donnay, "Disorder in a crystalline condensed phosphate"; W. S. MacKenzie, "The crystal-

line modifications of  $\text{NaAlSi}_3\text{O}_8$ "; G. W. Morey, "The system water-nepheline-albite: A theoretical discussion"; G. W. Morey, "The transition between the low- and the high-temperature form of sodium tripolyphosphate"; J. F. Schairer, "Melting relations of the common rock-forming oxides"; G. R. Tilton, G. L. Davis, G. W. Wetherill, and L. T. Aldrich, "Isotopic ages of zircon from granites and pegmatites"; O. F. Tuttle and N. L. Bowen, "The origin of granites in the light of experimental studies in the system  $\text{NaAlSi}_3\text{O}_8$ - $\text{KAlSi}_3\text{O}_8$ - $\text{SiO}_2$ - $\text{H}_2\text{O}$ "; H. S. Yoder and Th. G. Sahama, "Olivine X-ray determinative curve."

### SUMMARY OF PUBLISHED WORK

(1255) Variations in X-ray powder diffraction patterns of plagioclase feldspars. J. R. Smith and H. S. Yoder, Jr. *Am. Mineralogist*, 41, 632-647 (1956).

The angular separation between the  $(\bar{1}31)$  and  $(131)$  reflection in X-ray diffractometer patterns of 66 chemically analyzed natural plagioclases, 11 plagioclases synthesized in the dry way, and 4 plagioclases synthesized hydrothermally has been measured and plotted against composition. By this criterion, plagioclases synthesized in the dry way and natural plagioclases from thick stratiform mafic intrusions constitute two distinctly different series, each of which is closely defined by a single curve. Natural plagioclases from volcanic and hypabyssal rocks and plagioclases synthesized hydrothermally are intermediate between the two series. Other natural plagioclases, some of which have been assumed by other workers to belong to a "low-temperature" series, do not belong to either of the series mentioned above, and cannot be represented by any single curve. It is concluded that composition determinations cannot be made by means of the available curves based on the variation of reflection separations, because there is no a priori way of knowing how closely a given plagioclase is represented by a particular curve. Given the composition of a plagioclase, however, the curves are useful for making an estimate of its degree of inversion toward some undefined low-temperature state.

(1256) Paleobiochemistry. P. H. Abelson. *Sci. American*, 195, 83-92 (1956).

A variety of types of organic substances have been preserved in their original form or in only slightly altered state for many millions of years. Some of these occurrences are described, and their significance to potential knowledge of past living forms is pointed out.

(1257) *Petrographic modal analysis*. F. Chayes. New York, John Wiley & Sons, Inc. x + 113 pp. 1956.

A manual, intended for graduate students and advanced undergraduates, outlining the geometrical basis of the procedure, describing experimental studies of various kinds of analytical and sampling errors, and discussing a method of estimating and controlling the effect of grain size on experimental error in studies of two-feldspar granites.

(1258) The Holmes effect and the lower limit of modal analysis. F. Chayes. *Mineral. Mag.*, 31, 276-281 (1956).

Thin-section analysis is essentially an areal measurement, the measurement area usually being the upper surface of the section. If transmitted light is used for the measurement, the apparent areas of opaque grains are in general somewhat larger than their true areas on the measurement surface. For strictly spherical opaque particles in a transparent matrix the expected excess of apparent over true area is shown to be  $(\pi r^2 k)/(2r + k)$ ,



where  $r$  is the spherical radius and  $k$  is the thickness of the thin section. A table shows the relation between true and apparent area as a function of  $r/k$ .

- (1259) Pressure-temperature curves in some systems containing water and a salt. G. W. Morey and W. T. Chen. *J. Am. Chem. Soc.*, 78, 4249-4252 (1956).

A novel method for the determination of vapor pressures of saturated solutions has been applied to binary systems containing water and the salts LiF, NaF, KF, NaCl, KCl, RbCl, CsCl, PbCl<sub>2</sub>, Li<sub>2</sub>SO<sub>4</sub>, Na<sub>2</sub>SO<sub>4</sub>, K<sub>2</sub>SO<sub>4</sub>, Ti<sub>2</sub>SO<sub>4</sub>, Na<sub>2</sub>CO<sub>3</sub>, K<sub>2</sub>CO<sub>3</sub>, Na<sub>2</sub>O·4B<sub>2</sub>O<sub>3</sub>, Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>, and K<sub>4</sub>P<sub>2</sub>O<sub>7</sub> at some or all of the temperatures 374°, 400°, 500°, 600°, and 700° C.

- (1260) Experimental and theoretical studies of the mica polymorphs. J. V. Smith and H. S. Yoder, Jr. *Mineral. Mag.*, 31, 209-235 (1956).

An experimental and theoretical study has been made in order to determine the number and the structure of the possible polymorphs, and the structural relations between them. The simplest structures are 1M, 2M<sub>1</sub>, 2M<sub>2</sub>, 3T, 2O, and 6H polymorphs, and more complicated types can be developed. Some of the previously described polymorphs were not contained in the theoretical list and were re-examined. The 6M structure was found to be a 2M<sub>2</sub> polymorph, the 6-layer triclinic type was found to be a 2M<sub>1</sub> polymorph, and the 3M structure was shown to be a 3T type. The 24-layer triclinic structure could be described on a simpler 8-layer cell. This type together with a new 12-layer monoclinic structure, as well as other structures of higher periodicity, presumably consists of complex stacking and results from spiral-growth mechanism. Two extreme types of layer-disordered crystals may be built, and a disorder of individual ions may also occur. Single stacking faults result in twinned crystals. A new twin relation (180° rotation about the [100] axis) has been recognized. Twenty specimens from extreme geological environments have been examined to evaluate the control of environment on the stacking. The type of stacking could not be attributed solely to the influence of pressure and temperature. Composition seems to play a dominant role

in the type of stacking, and semiquantitative structural arguments appear to support this contention. The influence of growth mechanism is discussed. A scheme for the identification of the mica polymorphs by X-ray powder and single-crystal methods is given.

- (1261) The chemical formula of empressite. G. Donnay, F. C. Kracek, and W. R. Rowland, Jr. *Am. Mineralogist*, 41, 722-723 (1956).

Ag<sub>5</sub>Te<sub>3</sub> is synthetic empressite. Ag<sub>5-x</sub>Te<sub>3</sub> is the formula deduced, for the mineral, from cell dimensions and density of analyzed crystals.

- (1262) A provisional reclassification of granite. F. Chayes. *Geol. Mag.*, 94, 58-68 (1957).

The term "granite" could be usefully reserved for massive or weakly oriented plutonic rocks of color index less than 20 per cent and quartz content between 20 and 40 per cent by volume. A symbolic classification of rocks meeting these requirements is presented. The various classes are based on the relative proportions of plagioclase and alkali feldspar, and subclasses based on the ternary dominance ratio quartz-alkali feldspar-plagioclase are also proposed. The symbols are simple and easily remembered. A few of the common names now in use are retained for class designations.

- (1263) Organic constituents of fossils. P. H. Abelson. *Geol. Soc. Am. Mem.* 67, pp. 87-92 (1957).

This chapter describes the occurrence of amino acids in recent shells and in a variety of fossils as old as 360 million years. These findings are correlated with laboratory tests of the thermal stability of alanine.

- (1264) High-order transitions in (Na,K)AlSiO<sub>4</sub>. G. Donnay. Third International Meeting on Reactivity of Solids, Madrid, April 1956. 1957.

According to Ehrenfest's definition, a high-order transition corresponds to a discontinuity in any one of the derivatives of the Gibbs free energy  $G$ . The order of the transition is set equal to the order of the lowest derivative of  $G$  that shows a discontinuity. High-order

transitions are usually detected by observing an anomalous change in specific heat with temperature for a given composition. Nepheline,  $\text{Na}_{8-x}\text{K}_x\text{Al}_8\text{Si}_8\text{O}_{32}$ , is an example of a low-temperature phase in which changes of the composition  $x$  in a solid-solution range result in high-order transitions at  $x = \sim 1/4$  and  $x = 2.00$ . A singularity in the curve of cell volume  $V$  against  $x$  corresponds to a discontinuity in  $(\partial V/\partial x)_T$ , which is equal to  $(\partial^2 G/\partial P \partial x)_T$ . Such a transition is therefore of the second order.

(1265) Annual report of the Director for 1955-1956.

(1266) Optical analyzer. G. Donnay and J. D. H. Donnay. *Rev. Sci. Instr.*, 28, 145 (1957).

Two Polaroids, coupled for synchronous rotation, are mounted between glass plates and placed one at the top, the other at the bottom of a brass cylinder, which can rotate in a brass sleeve. The crystal on the goniometer head can be introduced into the cylinder through a hole in the sleeve and a corresponding one in the cylinder. The crystal can thus be observed between crossed Polaroids while it is rotated about the axis of the goniometer head.

(1267) The nepheline-kalsilite system: I. X-ray data for the crystalline phases. J. V.

Smith and O. F. Tuttle. *Am. J. Sci.*, 255, 282-305 (1957).

X-ray data are given for the following phases: high- and low- carnegieite, high- and low-nepheline, kalsilite, orthorhombic  $\text{KAlSiO}_4$ , synthetic kaliophilite, natural kaliophilite, anomalous natural kaliophilite, tetra-kalsilite, and  $\text{O}_2$ . Comparison of the cell dimensions indicates that the structures of all the phases except carnegieite are based on a tridymite-type framework. Synthetic natural and anomalous natural kaliophilite are not identical, but comparison of their X-ray properties indicates that they are structurally related. The variation of the cell dimensions of nepheline and kalsilite solid solutions has been determined. Discontinuities in the curves of composition versus cell dimensions are discussed in terms of the known crystal structure.

(1268) The solubility of solids in gases. G. W. Morey. *Econ. Geol.*, 52, 225-251 (1957).

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<sup>3</sup> Appointment from July 1, 1956.

<sup>4</sup> On leave of absence from October 1, 1956.

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<sup>13</sup> Appointment terminated November 30, 1956.

<sup>14</sup> Appointment from September 4, 1956.

<sup>15</sup> Appointment terminated July 12, 1956.

<sup>16</sup> Appointment terminated April 23, 1957.

<sup>17</sup> Appointment from April 15, 1957.

# DEPARTMENT OF PLANT BIOLOGY

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## INTRODUCTION

The Department of Plant Biology has been concerned for many years with the state of chlorophyll as it occurs in the live plant. This old problem, so far from being solved but long recognized as basic to an understanding of how chlorophyll functions in photosynthesis, has continued to be the center of interest of the Biochemical Investigations group of the Department. Most of the work in progress relates to the nature and mode of action of chlorophyll. In past years several approaches have been tried. The attempts to isolate the photochemically active components of disintegrated chloroplasts undertaken here ten years ago showed that reaggregation of the finely dispersed fragments restored activity. This work, however, did not lead to a solution of the problem of the chemical nature of *in vivo* chlorophyll. The chloroplast fragments used then were too large to permit the isolation of the chlorophyll complex in pure form. Considerably more success has been achieved in recent years by the characterization and partial purification of the protochlorophyll complex from leaves grown in the dark. The chlorophyll-protein complex formed by illumination of etiolated leaf material, being soluble, is more suitable for certain chemical studies than the natural chlorophyll complex obtained from dark green plants. The chlorophyll complex in fully developed leaves, however, differs from the protochlorophyll complex, or the chlorophyll complex made from it by exposure to light, in containing far more chlorophyll per unit protein. Therefore it is important to investigate not only etiolated plants with their water-soluble chlorophyll complex but also normal green plants that have a much higher chlorophyll content. With normal green plants the chlorophyll is generally in larger insoluble particles so that direct chemical isolation procedures cannot be used; indirect methods not based on chemical isolation seem more promising.

One of the obvious differences between extracted and natural chlorophyll is the wavelength position of the red absorption maximum. The absorption bands of extracted chlorophyll in organic solvents are at 10 to 20 m $\mu$  shorter wavelengths than those of natural chlorophyll in the plant. This shift is presumed to be due to the different states of combination of the chlorophyll, either with a protein in the live cells or with the solvent in extracts. One of our present objectives is to study in detail the shape of the red absorption band of chlorophyll *in vivo* and to see how it may be influenced by various procedures. This work has become possible through the development of a derivative spectrophotometer. The red band of chlorophyll in live material appears to consist of two components having a wavelength difference of about 10 m $\mu$ . These two components have not been separated chemically, but their presence is indicated by the shape of the derivative of the spectral absorbance curve.

Recent Russian experiments have shown that it is possible to shift the wavelength peak position of chlorophyll absorption by various treatments of the plant or of water extracts containing the chlorophyll-protein complex. These effects are presumably due to shifts from a form of chlorophyll absorbing at longer wavelengths to one absorbing at shorter wavelengths. A correlation between these experiments and the work in progress in this laboratory should eventually lead to a clearer understanding of the nature of the chlorophyll complex and the basis for its apparent occurrence in two different forms.

Attempts to isolate the naturally occurring protochlorophyll holochrome were continued during the year by Dr. Smith, who explored other methods of fractionating the leaf extracts. The earlier reported purification of about 75 per cent has not been exceeded, however. Fortunately, a newly used plant, taro, has been found to



give much larger etiolated leaves than had previously been attainable.

Another approach to the study of the natural chlorophyll-protein complex is to separate the protein and the chlorophyll, then to recombine them. Methods that result in restored photochemical activity of the reconstituted chlorophyll-protein complex have been developed by Dr. Vishniac at Yale University. They were explained to our group while Dr. Vishniac was here as a visiting investigator. During this time he found that purified chlorophyll *a* can be used in place of the alcoholic leaf extract, containing other substances as well as chlorophyll, with which his earlier experiments had been done.

Closely connected with the problem of the nature of the chlorophyll-protein complex and the mechanism of the formation of chlorophyll from protochlorophyll is the question why some mutant albino plants that produce chlorophyll do not stabilize it against bleaching by light. Additional observations relating to this question have been made during the year by Dr. Smith, using corn mutants of various types. Some of these mutants contained chlorophyll without phytol; others contained the chlorophyll combined with phytol. Mutants of both types were found to form chlorophyll on illumination but to lose it rapidly on further exposure to light. Therefore, the presence or absence of phytol cannot in itself be a determining factor for albinism.

Since the chemical structures of phytol and of carotenoids are similar, it had been thought that the phytol group of chlorophyll might come from carotenoids. This year, however, a carotenoid-free corn mutant was found that contains the chlorophyll in its phytolated form. It is therefore clear that phytol must arise from some other source than the carotenoids.

Since the evidence for the difference in the chemical nature of native and extracted chlorophyll is based largely on spectroscopic measurements, it is extremely important to have reliable methods for meas-

uring the absorption spectrum of pigments in living cells which so strongly scatter as well as absorb the light being measured. Dr. Latimer's work during the past year has shown that large errors can be made in determining the peak position of absorption bands of pigments that occur at high concentration in living cells. The errors may themselves be as large as the wavelength difference of peak absorbance between natural and extracted chlorophyll.

Dr. Latimer has investigated the nature of these discrepancies, which appear to be caused by selective scattering of the light by the groups of pigment molecules themselves. The scattering of light by the pigments changes very sharply with wavelength in the neighborhood of a pigment absorption band. By measuring the same suspension of *Chlorella* cells with a system collecting the light transmitted or scattered at different angles to the incident beam, Latimer found that the measured position of the absorption peak can vary by as much as 20 m $\mu$  with different experimental arrangements. None of the measuring arrangements, however, gave apparent peak positions for chlorophyll in live cells which were at as low a wavelength as that of chlorophyll in organic solvents. This work does not appear to invalidate the conclusion that natural chlorophyll is spectroscopically different from the extracted material, although these studies have raised grave doubt as to the validity of any comparisons of peak positions of the chlorophylls in live cells measured in different laboratories or even with different samples in the same apparatus. The results have also raised the question whether the double peak of chlorophyll absorption, observed by derivative spectrophotometry, is due to absorption by two actual components or whether one of these presumed components may be an artifact due to the dependence of light scattering on wavelength.

The study of the growth of algae and their pigment formation in a crossed gradient of light intensity on one axis, and of

temperature on the other, has been continued with several different algae.

The derivative spectrophotometer under development for some time has continued to take a large amount of time during the past year. Although minor improvements are still being made, this device is now in use for investigating the detailed shape of the red absorption band of chlorophyll in algal cells.

A large amount of original and highly significant work on aspects of photosynthesis close to the interests of the Department has been published in recent years in Russian, but only a part of this material had been obtainable in English translation. Mr. Milner has therefore translated about 130 articles, copies of which are being made generally available by The John Crerar Library in Chicago.

The group in Experimental Taxonomy has continued with its objective of exploring the mechanisms of natural selection and of evolution in several widely distributed groups of plants. Their efforts have, in the past, been directed toward the clarification of the evolutionary relationships between species and groups of species. Until recently this work was done largely by comparing the responses of identical plants grown in different environments, by extensive genetic studies, and by studying chromosome behavior in hybrid and parental forms of related species. Many of these long-range studies on plant relations have been completed, and the emphasis is now shifting to a study of the comparative physiology of closely related but ecologically distinct races. The present work is being carried out with some of the species whose genetic constitution and evolutionary history have already been determined. It is our hope to determine some of the specific physiological mechanisms that have given rise to the rich diversity among plant species.

Some progress in this direction has been made in the study of the comparative physiology of contrasting climatic races of the monkey flower, *Mimulus cardinalis*.

Current work on rates of respiration and photosynthesis demonstrates the suitability of this group of plants for such studies. Dr. F. J. F. Fisher grew seedlings of *Mimulus* in pure cultures. Using an infrared gas analyzer for carbon dioxide measurement, by the methods of Dr. John P. Decker, of the U. S. Forest Service at Tempe, Arizona, Fisher compared the rates of photosynthesis and respiration of flask cultures of several *Mimulus* strains. Very recently, through the generous cooperation of Dr. Decker, his apparatus is being utilized by him and by members of the staff for the study of *Mimulus* plants grown in pots in the greenhouse. The success of the gas analyzer in following accurately and rapidly the rates of respiration and photosynthesis under controlled temperatures and light intensities has established the feasibility of making comparative studies of fully developed plants as well as of seedlings in pure culture.

Concurrently with the physiological studies on the *Mimulus cardinalis* complex, genetic investigations and tests of growth responses at the altitudinal stations are being made to determine whether linkages exist between easily recognized morphological characters and the capacity of members of this group to survive at different altitudes. At Stanford the survival of segregating second-generation hybrid cultures in winter as compared with summer appears to be strongly correlated with conspicuous floral characters. If similar linkages exist with respect to the ability of segregating progeny to survive at the contrasting climates of the mountain stations, the interrelation between genetic and physiological mechanisms in natural selection should be clarified.

A comprehensive exploratory survey of the comparative growth and physiology of many species and races of Lemnaceae by Dr. Elias Landolt has been completed and is now in the process of publication. His experiments, carried on during 1954 to 1956 at this Department, at the Earhart Laboratory of the California Institute of



Technology in Pasadena, and at the Eidgenössische Technische Hochschule in Zürich, include studies on more than a hundred pure strains of various races and species of these duckweeds. Landolt measured growth rates under controlled experimental conditions and compared the data with extensive field observations at the original collection sites of many of the same strains that were studied as pure cultures. His experiments disclosed striking differences in growth rate among genera, species, and sometimes even between strains of the same species, at the same temperatures and light intensities. Some of these differences appear to be directly related to the ecological distribution of the various forms; others do not. Landolt's survey is an essential and important step in the further investigation of this unique family of aquatic plants which is widely distributed throughout the world.

For a number of years the relationships among various forms of yarrow have been under study by the group in Experimental Taxonomy. The recent success in obtaining first- and second-generation progeny between clearly distinct diploid species of central European origin suggests steps whereby the widely distributed and highly varied tetraploid and hexaploid forms found throughout the northern hemisphere may have arisen. A cross between the pink-flowered species *Achillea asplenifolia* and yellow-flowered *A. tomentosa*, both diploid and native in central Europe, yielded first-generation hybrid plants having white flowers that were nearly sterile. A few second-generation progeny were obtained, however, and among them are

many recombinations of the parental characters. The stabilization of such hybrid derivatives between diploid species through chromosome doubling may account for the origin of the extensive series of polyploid species and races found throughout Europe, Asia, and North America.

In the *Poa* investigations new data from screening tests involving forty-five apomictic hybrid lines of bluegrasses being grown in a wide range of climates in the United States and Europe show patterns of performance that can be clearly related to the characteristics of the parental species. The range of new recombinations that can be obtained from a given interspecific cross, although wide, does not ordinarily exceed the limits set by the parents. Within broad limits, predictions can therefore be made as to the kind of hybrid products to be expected from given interspecific combinations even in this highly complex group of plants characterized by multiple sets of chromosomes.

The selection of potentially useful agronomic forms of bluegrasses from the many hybrid combinations that have been produced has now been narrowed from more than forty to less than ten. Both the possibilities and the limitations of synthesizing new hybrid combinations of bluegrasses suitable for use in widely ranging climates now appear to be reasonably well established. The extensive studies on *Poa* are approaching a degree of maturity that has made the relationships among the different species more clearly understood. The completion of this study, begun in 1943, is anticipated within a few years.

## PERSONNEL

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Dr. Jens C. Clausen, who retired last year, attended a celebration in honor of Linnaeus's two hundred and fiftieth birthday at the Royal Agricultural College in Uppsala, where he received the honorary

<sup>2</sup> Resigned January 2, 1957.

## BIOCHEMICAL INVESTIGATIONS

## APPARENT SHIFTS OF ABSORPTION BANDS OF CELL SUSPENSIONS CAUSED BY OPTICAL EFFECTS

Paul H. Latimer

Absorption spectra of suspensions of cells are usually determined by measuring with a photocell the fraction of an incident beam of light transmitted by them. Turbid cell suspensions attenuate an incident beam in two different ways: by absorption, and by scattering part of the light out of the beam.

It is usually assumed that scattering varies gradually and uniformly with wavelength. If this were so, the absorption bands on spectral curves of cells would appear unaltered in shape and position, being merely superimposed on a uniform background caused by scattering.

We recently found that live cells containing high concentrations of pigments scatter light with a strong spectral selectivity. Sharp scattering maxima occur on

degree of Doctor of Agronomy in May 1957.

Dr. William M. Hiesey attended the Seventh International Grasslands Conference in New Zealand, and visited various New Zealand and Australian laboratories and field experiment stations engaged in grass breeding and plant physiology. He also served as President of the Western Society of Naturalists.

Dr. James H. C. Smith attended the International Congress of Photobiology at Turin, and visited numerous European laboratories concerned with photosynthesis.

Professor Wolf Vishniac, of Yale University, visited the Department for three weeks; he demonstrated his methods of separating chlorophyll from its protein, and then reconstituting the complex to a photochemically active form.

Dr. Theodosius Dobzhansky, of Columbia University, worked at the Mather field station during the summer of 1957 in continuation of his extensive genetic investigations on *Drosophila*.

the long-wavelength sides of the absorption bands. Since these findings contradict the usual assumption made in absorption spectroscopy of cell suspensions, we carried out experiments to determine whether this anomalous light scattering by cells could significantly influence ordinary measurements of their absorption spectrum.

Absorption spectra of a suspension of the green alga *Chlorella pyrenoidosa* were measured in two different ways, one designed to maximize, the other to minimize, the influence of scattering. Both measurements were made with a Beckman DK-2 recording spectrophotometer.

Three arrangements of part of the optical system of the spectrophotometer are pictured schematically in figure 1. Arrangement (a) is the usual one. The light is measured by a photomultiplier tube placed so far from the sample that it collects only light transmitted directly or scattered at



angles up to about  $\pm 3^\circ$ . Arrangement (b), a *hypothetical* modification of (a), would reduce the influence of scattering by permitting the photocell to collect all light scattered at angles up to about  $\pm 30^\circ$  to  $50^\circ$ . Arrangement (c), described by Shibata (Year Book 55, pp. 252-256), is equivalent to (b) with respect to the collection of scattered light and is experimentally advantageous. A diffusing plate, inserted between the sample and photocell, causes a small but *representative* sample of the transmitted and scattered light that strikes it to enter the photocell. Control experiments showed that any differences between

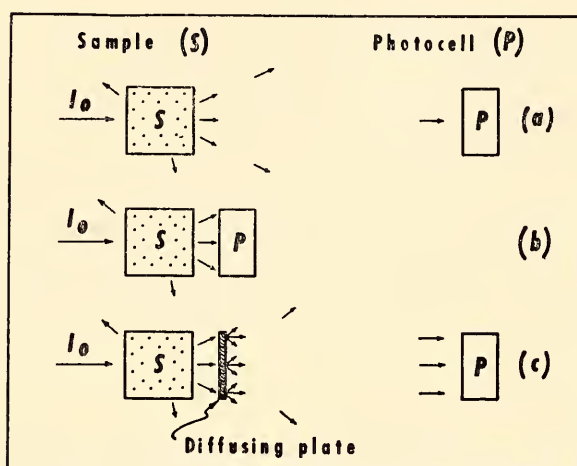


Fig. 1. Schematic diagrams of part of the optical system of a spectrophotometer.

spectral curves obtained with arrangements (a) and (c) must be due to the difference in the effective angle subtended by the photocell at the suspension.

Figure 2 shows the absorption curves of a suspension of *Chlorella* measured with arrangements (a) and (c), and also our previously published scattering curve of this organism for light scattered at  $90^\circ$ , but qualitatively representative of light scattered at most angles.

The strong influence of scattering on the upper absorption curve, (a), is apparent from its over-all height. Of particular importance are the differences of more than 10 m $\mu$  between the positions of the major bands on the two absorption curves. Parallel differences are found between similar

absorption curves of *Chlorella* published by Shibata et al. These authors, however, emphasized only the sharpness of the bands on curves measured with diffusing plates and did not draw attention to the difference in the positions of the peaks.

Curve (c) is in reasonably good agreement with other absorption spectra reported for *Chlorella*; presumably the positions and shapes of the bands on it are approximately correct.

A plausible explanation of the differences between curves (a) and (c) can be suggested in terms of the given scattering spectrum. For instance, the band on curve (a) at 687 m $\mu$  appears to represent the *sum* of the *scattering* band at 690 m $\mu$  and the *absorption* band at 675 m $\mu$ . We shall see later, however, that the explanation of these results must be more complex than is suggested by this simple picture.

Somewhat different results were obtained with *Chlorella* cells of the same strain but from other cultures. Although the red absorption maximum of cells from all cultures occurred at 675 m $\mu$  on the (c) curves, the position of this maximum on the (a) curves varied with the culture from 683 m $\mu$  to 691 m $\mu$ . The bands at the longest wavelengths were obtained with cells that appeared to be in the best physiological condition (in the rapid growth phase).

We also measured pairs of absorption curves of two suspensions of spinach chloroplasts, one of large particles, whole chloroplasts of average dimension 5 to 6  $\mu$ , the other of very small fragments. Results similar to those for *Chlorella* were obtained with the whole chloroplasts, although the difference in the positions of the band on the two curves was smaller. The chloroplasts were then broken by forcing them through a needle valve; centrifugation gave a clear green "suspension" of small fragments. In contrast to the results for whole chloroplasts, the bands of this suspension on absorption curve (a) were identical in shape and position with those on curve (c).

In order to learn more about the factors that produced the differences between the absorption curves in figure 2, we constructed a special spectrophotometer, figure 3, for measuring spectral curves of light transmitted or scattered at small

Some of the results for a suspension of *Chlorella* cells are given in figure 4. Directly transmitted light (at  $0^\circ$ ) gives an absorption curve with a band maximum at  $688\text{ m}\mu$ , in reasonably good agreement with its counterpart in figure 2, curve (a).

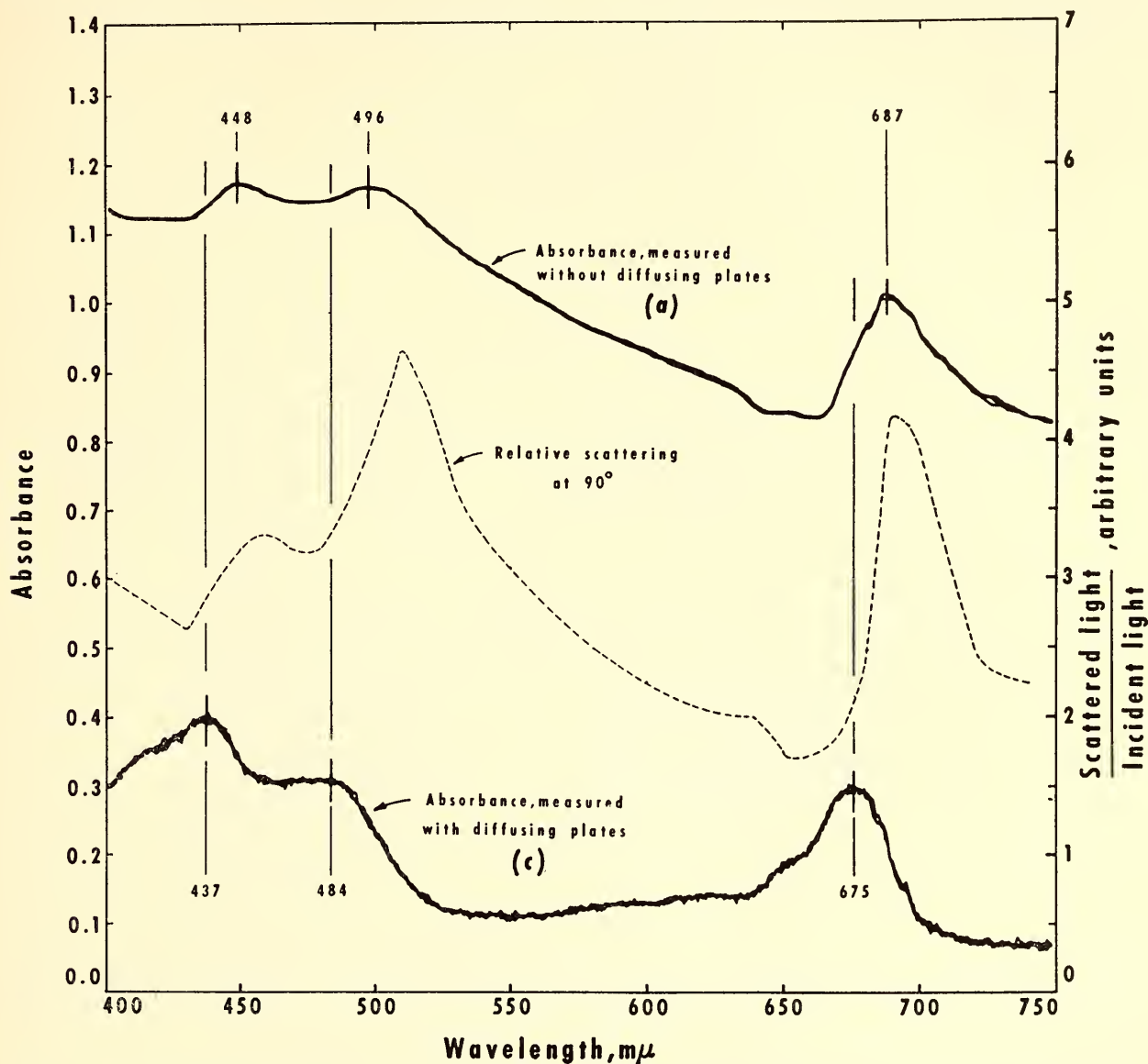


Fig. 2. *Chlorella*: absorption spectra measured with Beckman DK-2 recording spectrophotometer using arrangements (a) and (c) in figure 1. Both absorption curves are original spectrophotometer recordings, each made in triplicate. Center curve: scattering by *Chlorella* at  $90^\circ$ .

angles. Spectral curves were determined by comparing light transmitted by a blank vessel containing medium with that transmitted or scattered at certain angles by the suspension. For comparison, we also measured a spectral curve of the suspension with the same spectrophotometer using a diffusing plate as in arrangement (c), figure 1.

Light scattered at about  $3.4^\circ$  ( $\pm 0.5^\circ$ ), however, shows the band maximum to be at  $682\text{ m}\mu$ . On going out to  $32^\circ$  ( $\pm 8^\circ$ ), we find the band maximum to have shifted back to  $668\text{ m}\mu$ . By measuring the total light transmitted or scattered at all angles from  $0^\circ$  to about  $90^\circ$  with a diffusing plate, an absorption curve with a maximum at  $672\text{ m}\mu$  was obtained.



It should be noted in figure 4 that the absorbance scale for each angular curve is an arbitrary one. The measured values of percentage transmission for the angular curves were very small. They were normalized to give minimum values equal to that of the curve measured with the diffusing plate.

$m\mu$ , depending on the conditions of the measurement. The curve measured with a diffusing plate must be a composite of a number of spectral curves for light at different angles with band maxima at different wavelengths. The relation of this composite, made up of widely divergent components, to the actual absorption spec-

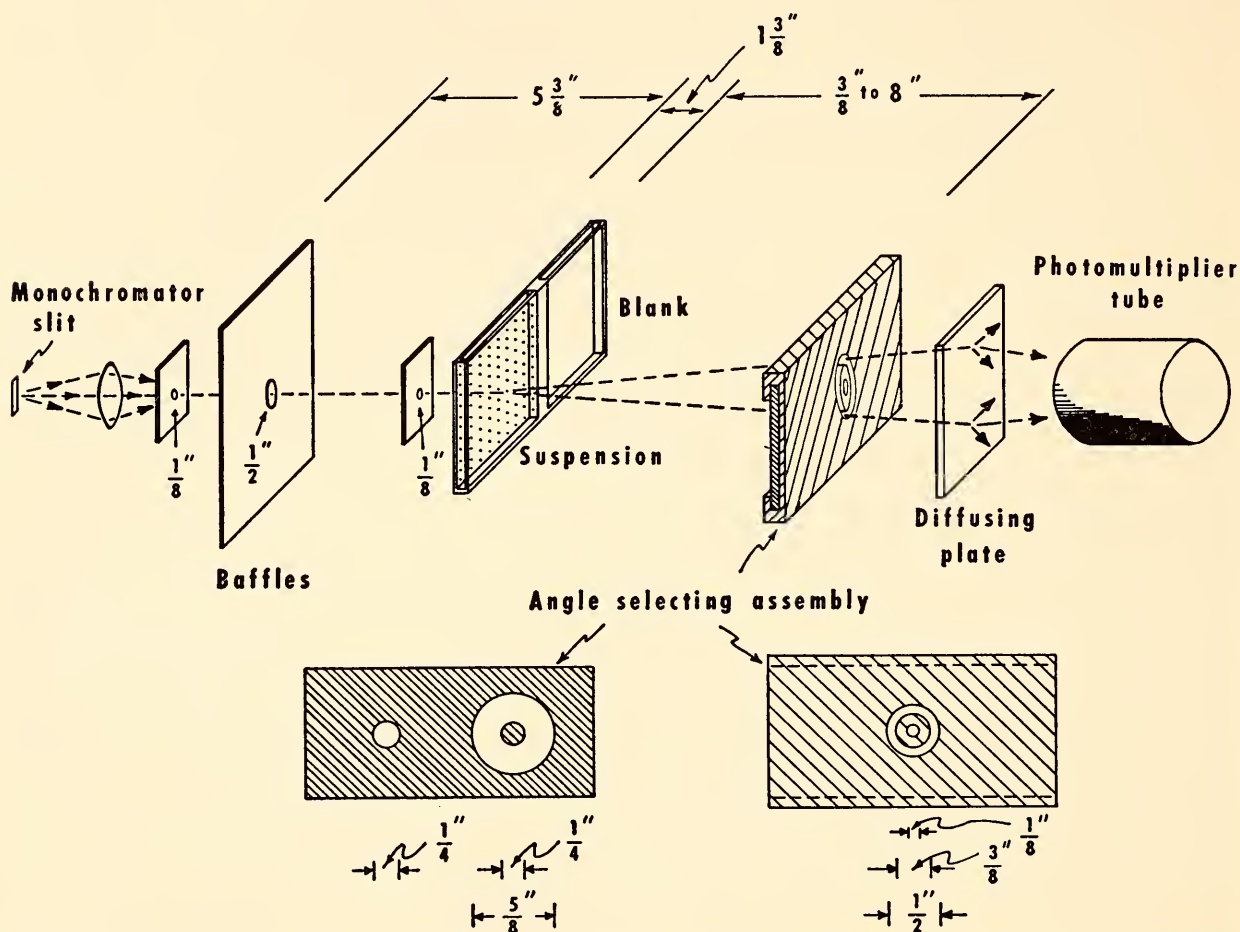


Fig. 3. Apparatus for measuring spectral curves of light transmitted or scattered by suspensions at small angles. The angle is varied by changing the distance from the suspension to the angle-selecting assembly.

This criterion for normalizing the curves is somewhat arbitrary. Although it permits the presentation of the data in a familiar form, it does lead to the apparent anomaly of negative absorbance values on the  $32^\circ$  curve. This can be explained in part by the fact that light scattered at  $32^\circ$  by the cells has no counterpart from the blank vessel which transmits all the light at  $0^\circ$ .

Figure 4 shows that, for the given cell suspension, the same absorption band which we presume to lie actually at  $672 m\mu$  appears at wavelengths from  $688 m\mu$  to  $668$

$m\mu$  of the cells does not seem to be immediately obvious.

Barer and others have suggested the use of a special suspension medium to minimize scattering by live cells and hence also its influence on measurements of their absorption spectra. By choosing a medium with an index of refraction equal to that of the cells, or the cell surfaces, scattering and the effects that it produces should be substantially reduced. In order to determine the influence of the medium on the effects of scattering we repeated the meas-

urements of figure 2 with *Chlorella* cells from a given culture in four suspension media (water and different water solutions of bovine serum albumin) having different indices of refraction.

main absorption band which appears at about 685 m $\mu$  for these cells in water,  $n=1.33$ , is found at 668 m $\mu$  for cells in a solution of about 40 per cent albumin ( $n=1.42$ ). The (a) curve for  $n=1.40$ ,

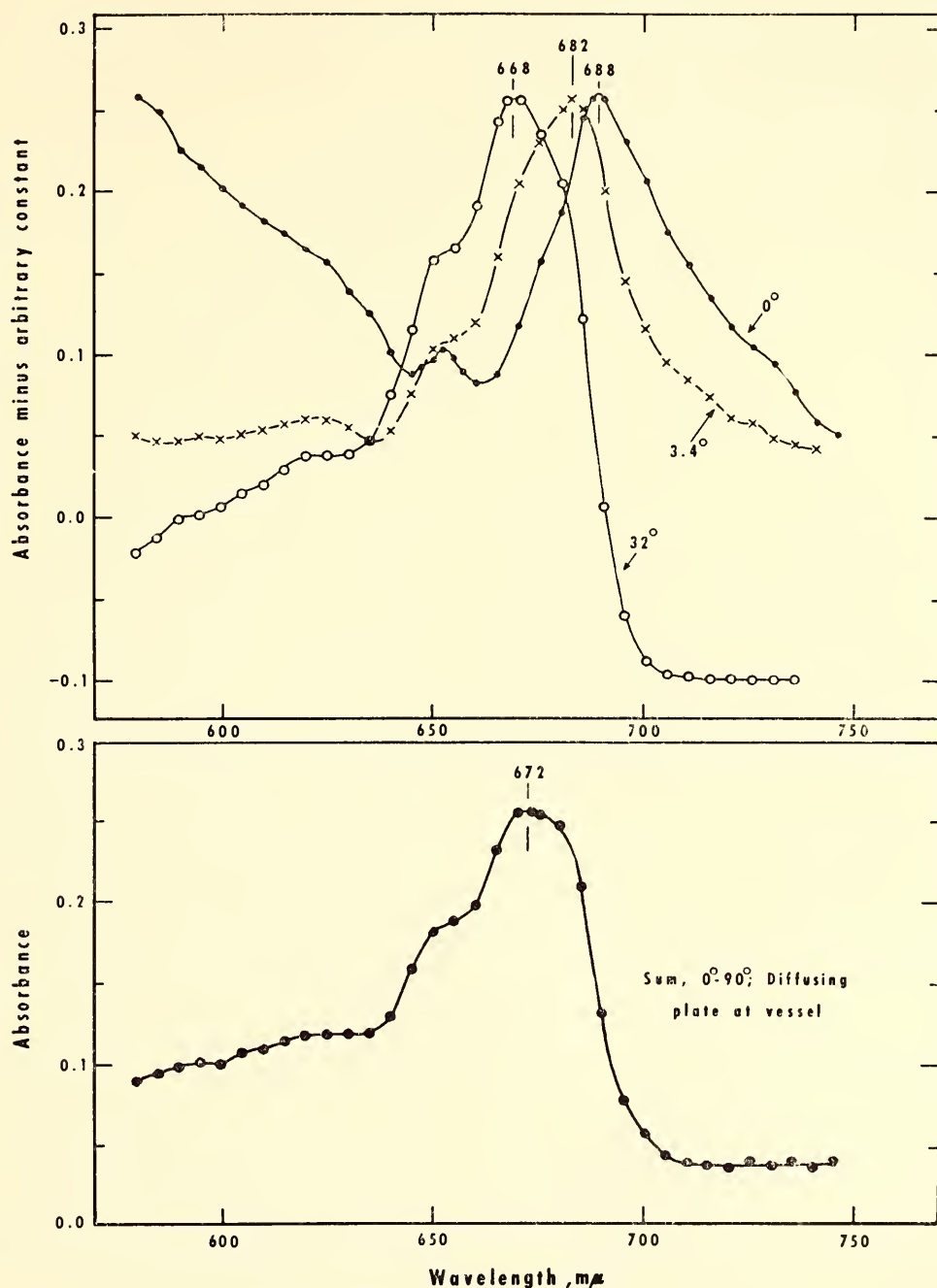


Fig. 4. *Chlorella*: absorption spectra determined by measuring light transmitted or scattered by a cell suspension at indicated angles with apparatus of figure 3. The lower curve was determined by removing angle-selecting assembly and placing diffusing plate behind vessel as in figure 1.

The spectral curves obtained with these suspensions are shown in figure 5. The curves measured with arrangement (c) vary only slightly with the suspension medium. The (a) curves, however, show striking variations. In the red region, the

which is approximately the index of refraction of the cells, most nearly resembles the (c) curves which presumably represent the actual absorption spectrum.

Whereas the results given in figures 2 and 4 could be explained simply in terms



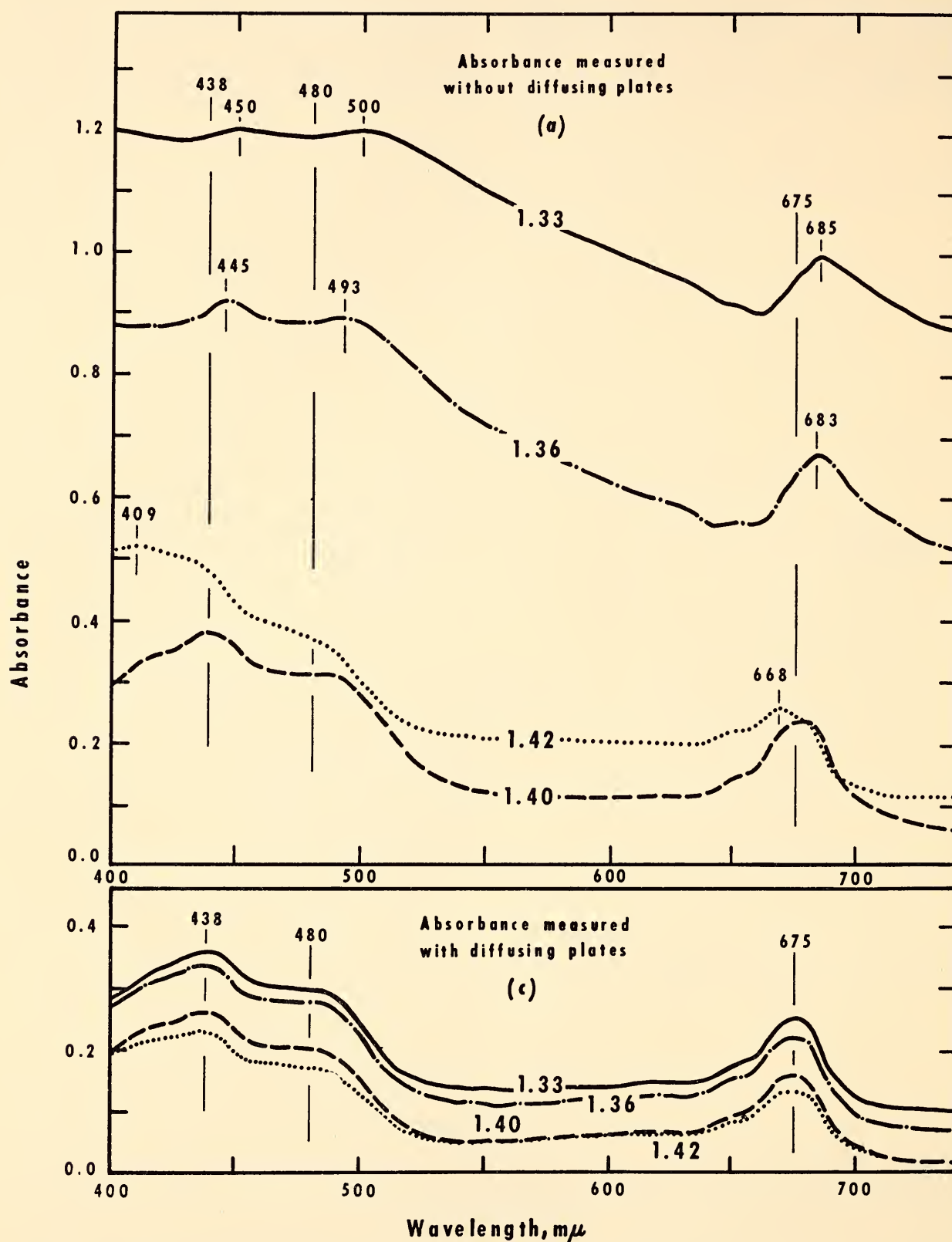


Fig. 5. *Chlorella*: absorption spectra of cells in different solutions of bovine serum albumin having indicated indices of refraction. Curves measured with Beckman DK-2 recording spectrophotometer using arrangements (a) and (c) in figure 1.

of selective scattering, this phenomenon does not appear to explain the results in figure 5. Here, changes in the index of refraction of the medium which is *not* in contact with the colored cell structures, the sources of selective scattering, caused striking changes in the apparent position of the absorption band. Therefore, it is necessary to look for another mechanism to explain our results.

Lothian and Lewis recently published absorption curves of red blood cells which were determined with spectrophotometer arrangements similar to (a) and (c) in figure 1. By measuring only light transmitted directly (at about  $0^\circ$ ), they obtained an absorption curve with a "valley" instead of a "peak" at the position of the Soret band. This result was explained in terms of light interference and diffraction produced by the individual red blood cells.

The diffraction effect can be demonstrated by the classic experiment of holding a penny in sunlight and examining the shadow; a bright spot caused by diffraction should be found at the center of the shadow. Changes in the size or opacity of the penny would change the whole pattern of illumination in the shadow. Presumably, the red blood cells act like semitransparent pennies, the transmission of which varies with wavelength. At the absorption maximum the effects of diffraction are greatest and produce a maximum instead of a minimum illumination in the "zero degree" direction. Actually, the high intensity of light "transmitted" by an opaque disk in the zero degree direction is compensated for by "abnormally" low intensities in certain other directions. Thus, when most of the light transmitted or scattered in various directions is collected by the photocell, as with arrangement (c), figure 1, the effects of diffraction should largely cancel out.

At first glance, it appears that this type of diffraction and interference effect could not explain our results. In general, the effect should be symmetrical about absorption bands whereas the effects that we ob-

served are not. Diffraction and interference by a semitransparent disk, however, depend on its index of refraction. Selective scattering, which was mentioned earlier, is apparently caused by changes with wavelength in the index of refraction of the cellular components. The changes, caused by anomalous dispersion, are *not* symmetrical about absorption maxima.

It is possible that the effects shown in the figures are caused by diffraction and interference as governed by anomalous dispersion. To test this hypothesis, we carried out preliminary calculations of the effects of diffraction and interference on curves of this type for cells in which anomalous dispersion is strong. The results support this interpretation of our curves, but the relation between this mechanism and the one suggested earlier in terms of selective scattering is not clear.

The experimental evidence indicates that in addition to absorption at least two types of optical phenomena occur in cell suspensions: selective scattering, and light interference. The implications of the presence of these phenomena are twofold.

Some of our ideas of the relation between absorption by pigments in live cells and the light transmitted by cell suspensions will need revision. It appears likely that many published absorption spectra have been at least slightly distorted by these optical artifacts.

Theoretical considerations suggest that optical studies such as those described here may be useful in obtaining information about the packing and arrangement of pigment molecules *in vivo*. Studies of scattered light have already provided valuable information about the size and shape of such simple particles as protein molecules, aerosol droplets, synthetic polymers, and particles in interstellar space. The optical effects described above, which depend on absorption, should be related to the submicroscopic arrangement of pigment molecules *in vivo*. Possibly the different results (of the type shown in fig. 2) which we obtained for *Chlorella* cells from different



cultures were due to inherent differences in the arrangement of pigment molecules in the different cells.

THE SHAPE OF THE RED ABSORPTION  
BAND OF CHLOROPHYLL IN  
LIVE CELLS

C. S. French and Helen S. Huang

It has long been believed that the difference in the wavelength position of the red absorption band of chlorophyll in live cells as compared with its position in the extracts of chlorophyll indicates a chemical combination of chlorophyll with some other material in the plant cell, presumably protein. Many observations have placed the peak position at different wavelengths, generally in the range 670 to 680 m $\mu$ . Recent work of Krasnovsky and of Sapozhnikov has shown that it is possible to shift the wavelength peak position of native chlorophyll both in cells and in disintegrated chloroplasts suspended in water. Treatments involving pH and temperature changes have been shown to influence the peak position.

Furthermore, in chlorophyll freshly formed from protochlorophyll, Shibata found that the peak position initially at 683 m $\mu$  is shifted rapidly by a dark reaction to about 672 m $\mu$  and then again slowly approaches longer wavelengths. Latimer has found that scattering may produce the apparent absorption maximum in *Chlorella* anywhere in the range from 665 to 690 m $\mu$ , depending on the way in which the measurements are made.

Halldal, analyzing the absorption spectra of algae, has found that two different chlorophyll *a* curves can be used in the red part of the spectrum in varying proportions to match the observed absorption curves recorded with the Beckman spectrophotometer by Shibata's opal-glass methods. The two curves by which Halldal fitted the measured spectra had their peaks at 670 and 682 m $\mu$ .

Recent work by derivative spectrophotometry has shown the apparent doubling of the chlorophyll *a* peak as in figure 6.

In this figure the first derivative of absorbance with respect to wavelength is plotted against wavelength for two samples. One of them is pure chlorophyll *a* in ether solution prepared by Dr. Smith. The other sample is *Chlorella pyrenoidosa* grown on an agar surface and measured by placing the agar layer directly in the spectrophotometer.

The point where the derivative curve crosses the zero line represents the peak of the corresponding absorbance curve. Chlorophyll *a* in ether has its peak at 663 m $\mu$ , and in this sample of *Chlorella* at 673 m $\mu$ . In *Chlorella* the whole curve is shifted to the right by about 10 m $\mu$  and is complicated by the presence of chlorophyll *b* as shown by the deep notch and the extra peak. Furthermore, just below the point where the derivative curve crosses the zero line there is a shoulder. This flat shoulder, which in some samples may occur at or above the zero line, indicates that the absorbance curve has a constant slope over a 5 m $\mu$  range. This type of curve is obtained for an absorption spectrum consisting of two overlapping components. An attempt was made to study the influence of temperature and light intensity on the shape of the derivative curve of the red band of chlorophyll *a* in *Chlorella* as indicated by this shoulder. Curves for the algae grown on different parts of the crossed gradient plate were indeed different. No apparent correlation existed, however, between the temperature or light intensity under which the algae had been grown and the shape of the derivative absorbance curves. In another experiment a suspension of *Chlorella* was put on agar surfaces at different concentrations. The observed peak position was at a somewhat shorter wavelength with the denser layers of algae, but density itself had very little influence on the shape of the curve.

Many species do not have the apparent doubling of the chlorophyll *a* peak as shown by the shoulder in the derivative curve. The red alga *Porphyridium aeru-*

*gineum*, studied in collaboration with Dr. Haxo, showed a single smooth curve indicative of only a single type of chlorophyll *a* in this organism. The curve for a white

that for *Chlorella*, was found in the alga *Cyanidium*. In *Anacystis*, studied with Dr. Halldal, a small shoulder was found in the algae grown at low intensity and high

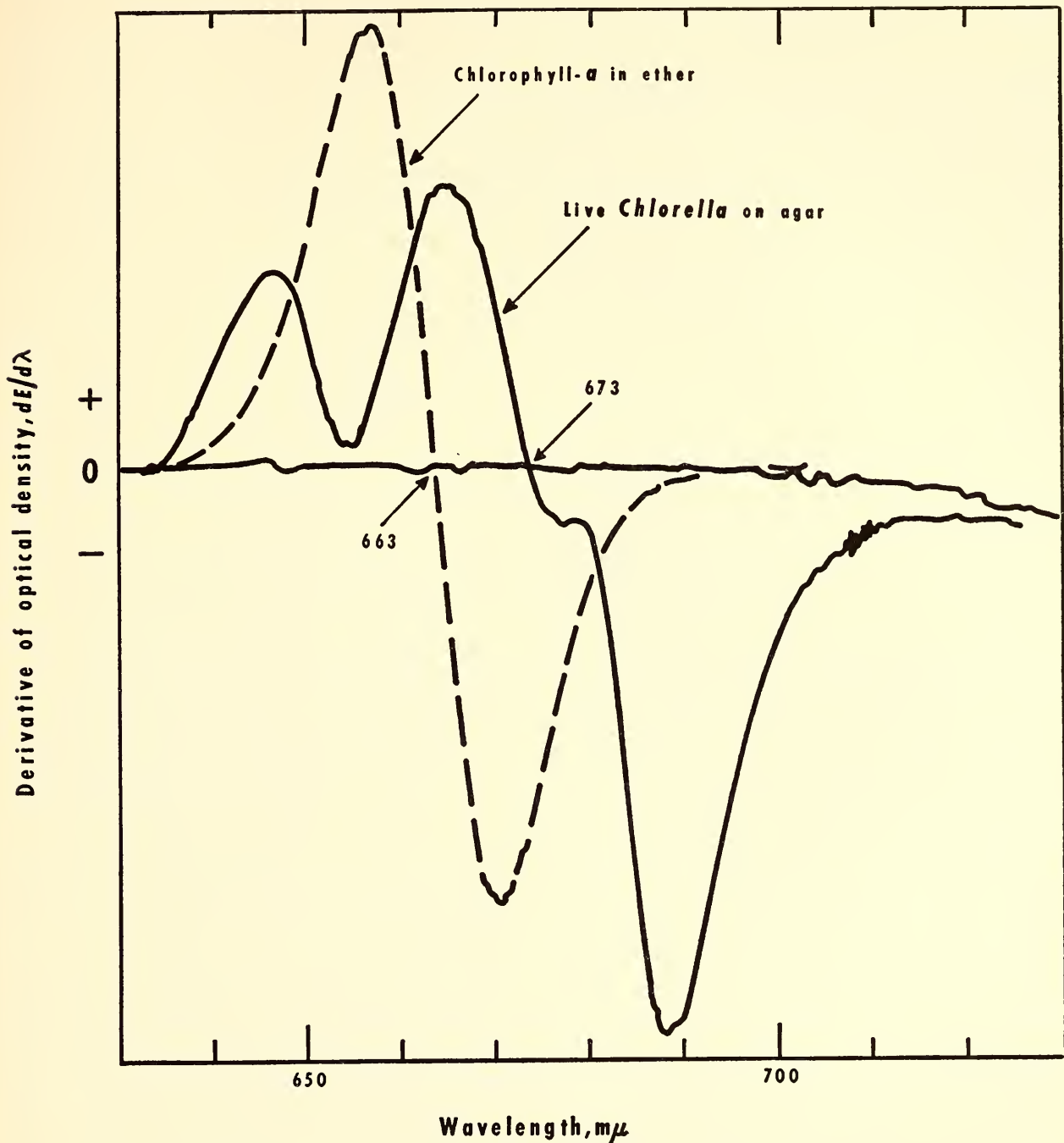


Fig. 6. Derivative spectra of a chlorophyll-*a* solution compared with that for the alga *Chlorella*. In *Chlorella* the presence of two chlorophyll-*a* components is shown by the shoulder at 680 mμ. Chlorophyll *b* contributes the peak and dip at the left.

ivy leaf containing only a very small amount of chlorophyll showed a slight shoulder. Curves for various other leaves have shown this apparent doubling of the chlorophyll *a* peak in different proportions. A slight shoulder, much less evident than

temperature on the gradient plate and in a culture at room temperature in daylight.

It is not yet certain whether this apparent doubling of the chlorophyll *a* peak is due to two real chlorophyll *a* components or whether one of them may be the long-



wavelength scattering peak discussed earlier in this report by Latimer.

PHOTOCHEMICAL ACTIVITY OF RECONSTITUTED CHLOROPHYLL-PROTEIN COMPLEXES

Wolf Vishniac

Previous experiments (W. Vishniac, in H. Gaffron, ed., *Research in Photosynthesis*, Academic Press, New York, 1957) indicated the possibility of obtaining cell-free soluble preparations from chloroplast fragments which would carry out light-dependent reductions of triphosphopyridine nucleotide (TPN). These experiments were continued at the Department of Plant Biology.

**Methods.** Photochemical activity was measured in two ways: a drop in redox potential as determined with the platinum electrode, and the reduction of TPN as determined by the reduction of oxidized glutathione (GSSG) to glutathione (GSH) in the presence of a TPN-linked GSSG reductase. The reaction vessel (fig. 7) accommodated platinum and calomel electrodes and could be flushed with helium or air, so that the reaction mixture was stirred by the passage of gas. At the conclusion of the experiment the reaction mixture was sucked into the sampling flask for analysis. The entire assembly was submerged in a constant-temperature bath at 10° and could be illuminated with an incandescent bulb at about 4000 foot-candles. The output of the platinum-calomel couple was balanced by a potentiometer and fed into a recorder.

GSH was determined by the method of Dische and Zil (*Proc. Assoc. Research Ophthalmol.*, 19, 104 [1950]). GSSG reductase was prepared from yeast according to Racker (S. Colowick and N. O. Kaplan, eds., *Methods in Enzymology*, Academic Press, New York, 1955, vol. II, p. 723). Chlorophyll *a* was prepared under the direction of Dr. J. H. C. Smith. In some experiments a purified chlorophyll preparation, kindly supplied by Dr. S. Granick, was used which was largely chlorophyll *a*.

"Mixed leaf carotenes" had been prepared by Dr. H. H. Strain.

**Preparation of active material.** Chloroplast fragments from spinach leaves were prepared as previously described (cf. P. R. Gorham, in S. Colowick and N. O. Kaplan, *Methods in Enzymology*, Academic Press, New York, 1955, vol. I, p. 22). The green paste was ground in a Waring Blender at -5° to -10° with 10 volumes of prechilled acetone for 3 minutes and filtered, and the sediment was re-extracted

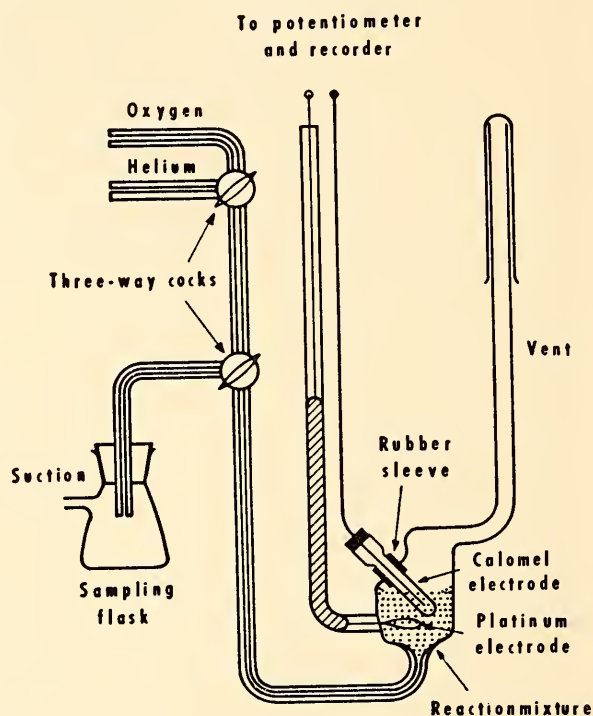


Fig. 7. The reaction vessel. A test tube over the vent prevents back diffusion of air.

twice more with cold acetone. The acetone powder thus obtained is distinctly green, even though most of the chlorophyll has been extracted; it retains its activity when stored for an appreciable time if kept cold, dark, and dry. The acetone powder is extracted with 5 volumes of 0.05 M potassium phosphate, pH 7.0, for 10 to 20 minutes with slow stirring or, alternatively, by working the mixture in a small Tenbroeck homogenizer in the cold. Upon centrifugation (20,000 × g) the supernatant fluid ("extract") is yellowish or light brown but not green, and the absorption spectrum reveals no chlorophyll peaks. The sedi-

ment ("residue") contains all the green color of the acetone powder.

final suspension was divided into two 5-ml portions. One portion was centrifuged

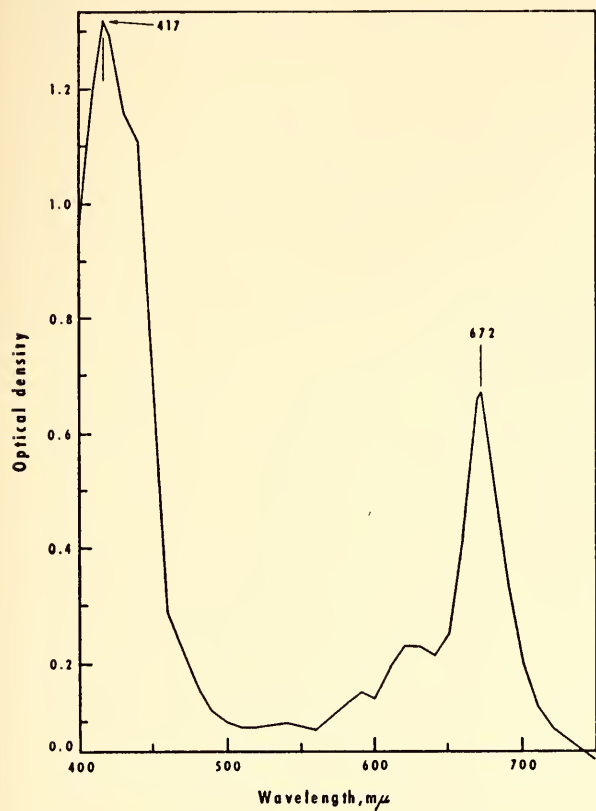


Fig. 8. The absorption spectrum of cold-acetone-extracted chloroplasts after removal of the water-soluble components. Since the material was essentially free from carotenoids and water-soluble pigments, this curve represents the absorption of protein-bound chlorophyll.

A spectroscopic examination of the residue was carried out as follows: 200 mg of acetone powder was suspended in 10 ml of water and exhaustively extracted by six centrifugations, resuspensions in water, and grinding with a homogenizer. The

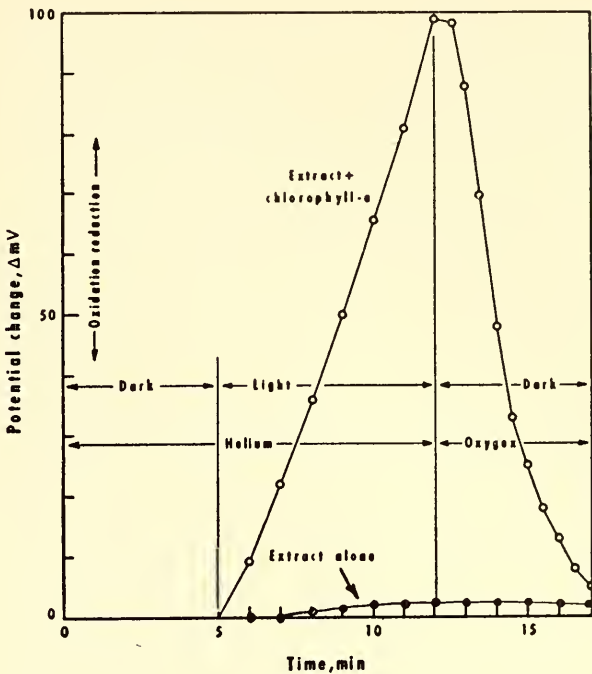


Fig. 9. Formation of photoreducing power by a water-soluble protein extract of chloroplasts when chlorophyll is added.

and extracted three times with 95 per cent ethanol, and the brownish sediment was resuspended in 5 ml of water. This decolorized material served as a blank against which the absorption spectrum of the suspended residue in the other 5-ml portion was read. The spectrum (fig. 8) showed peaks at 417 and 672 mμ. Therefore no chlorophyll was added in experiments in which the activity of the residue was tested.

*Experiments.* Acetone powder (1.0 g) was extracted with 5.0 ml of phosphate

TABLE 1. The Increase in Optical Density and the Wavelength Shifts of the Peaks of a Solution of Chlorophyll When Added to a Large Volume of Dilute Phosphate Buffer

Shift of Red Peak			Shift of Blue Peak		
Time after Mixing, min	Maximum Absorption, mμ	Optical Density	Time after Mixing, min	Maximum Absorption, mμ	Optical Density
1.8	668	0.420	4.0	429	0.750
5.00	669	0.435	7.0	431	0.740
10.4	672	0.467	12.3	435	0.715
15.5	676	0.492	17.5	442	0.670
36.5	685	0.520	42.0	449	0.615



buffer. Of the supernatant fluid, 1.0 ml was placed in the reaction vessel in the dark, under a stream of helium. When chlorophyll was added, 0.05 ml. of a 0.78 mg/ml solution in ethanol was used. Controls without chlorophyll contained an equal amount of ethanol. Figure 9 shows a

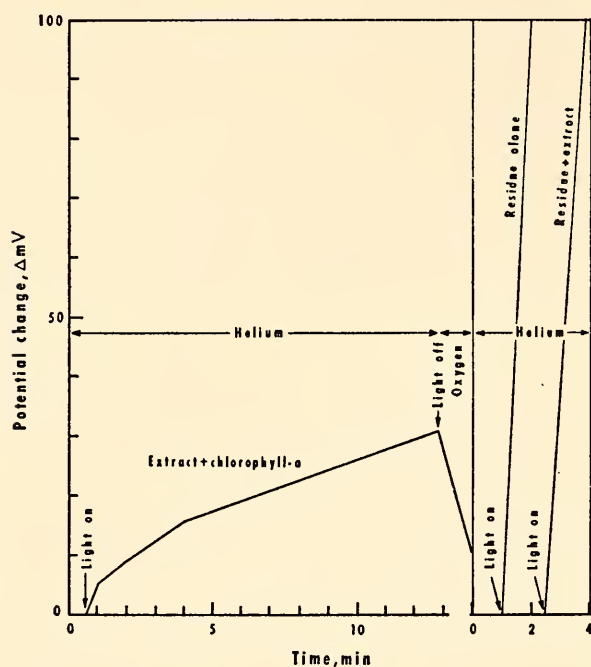
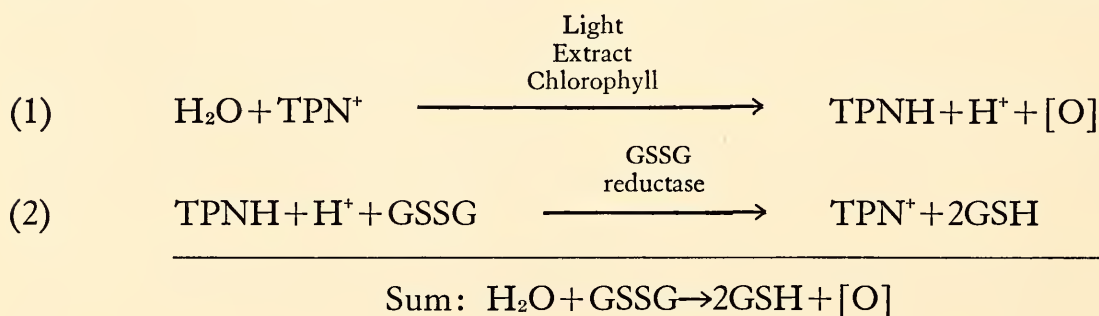


Fig. 10. The potential changes in the extract + chlorophyll as compared with the residue not extractable by water from the cold-acetone-treated chloroplast material. The residue is more active than the extract as measured by potential changes but less so as measured by chemical change as in table 2.

The addition of a small volume of ethanolic chlorophyll solution to water or phosphate buffer results in a colloidal dispersion of the chlorophyll, so fine that the suspension appears clear to the eye. Within 30 minutes, however, the green suspension turns turbid, presumably because of the coagulation of chlorophyll into larger aggregates. The spectroscopic changes that occur when 0.05 ml of chlorophyll (0.8 mg/ml) in ethanol is mixed with 2.0 ml of phosphate buffer were measured by Dr. C. S. French. The results (table 1) can be interpreted as a change in selective scattering caused by changing particle size.

One attempt was made to reduce TPN: 30  $\mu$ M of TPN was added to the extract and chlorophyll; the mixture was incubated in the light under helium for 20 minutes and deproteinized, and the supernatant fluid was examined for absorption at 340 m $\mu$  by reduced TPN. No significant absorption was found that could be ascribed to TPNH. The incubation of catalytic amounts of TPN, GSSG reductase, GSSG, extract, and chlorophyll, however, revealed a light-dependent reduction of GSSG that was absolutely dependent on TPN. Hence the reduction of GSSG must have been mediated by the prior reduction of TPN, and can be formulated as follows:



representative experiment from which the effect of light and chlorophyll is evident, also the oxidation that takes place when the helium in the atmosphere is replaced by oxygen. In one experiment, but not in later trials, the addition of 0.1 ml of a saturated solution of mixed leaf carotenes in ethanol further increased the rate of change of redox potential. Boiled or frozen extracts were inactive.

The nature of the oxidized product, [O], is unknown. The availability of a soluble system that can catalyze reaction 1 opens the possibility of an enzymological analysis of photosynthesis. The results of this experiment are found in figure 10 and table 2. It can be seen that there is a consistent increase in GSH in the light. The data suggest, but do not show conclusively, a supra-additive effect when extract and resi-

due are combined. To judge by specific activity, most TPN-reducing activity resides in the extract, and most of the electrode activity in the residue.

TABLE 2. Comparison of Specific Activities of Extract and of Residue for Formation of Photoreducing Potential and for TPN-Coupled Glutathione Reduction

	Reaction Systems		
	Extract	Residue	Extract + Residue
Components			
Buffer, ml . . . . .	1.0	1.0	0.0
Extract, ml . . . . .	1.0	0.0	0.0
Residue suspension, ml . . . . .	0.0	1.0	1.0
GSSG reductase, ml. . . . .	0.1	0.1	0.1
GSSG, 0.1 M, ml. . . . .	0.1	0.1	0.1
TPN, 10 <sup>-3</sup> M, ml. . . . .	0.1	0.1	0.1
Chlorophyll <i>a</i> , soln., ml . . . . .	0.05	0.0	0.0
Protein, mg . . . . .	5.9	37.9	43.8
Activity			
Change in emf			
Initial rate, Δmv/min, in light . . . . .	10	130	130
Specific activity, Δmv/min × mg protein, in light. . . . .	1.69	3.4	3.0
GSSG reduction			
μM GSH in light. . . . .	3.92	1.79	4.95
μM GSH in dark. . . . .	2.63	0.52	1.79
ΔμM GSH . . . . .	1.29	1.27	3.16
Specific activity, ΔμM GSH/mg protein . . . . .	0.22	0.034	0.072

A FAT-SOLUBLE COMPONENT OF CHLOROPLASTS NECESSARY FOR PHOTO-CHEMICAL ACTIVITY

Max Milner

The observation reported by Lynch and French in Year Book 55 that β-carotene appears to reactivate the Hill activity of ether-extracted chloroplasts has prompted additional studies to determine whether this effect might be provided by other ether-soluble compounds as well.

Techniques somewhat more quantitative than those used heretofore have been developed, by means of which the β-carotene effect has been confirmed, but only with much larger quantities of carotene than were previously used. With lyophilized preparations of both chard and pokeweed chloroplasts, virtually complete elimination of Hill activity has been achieved with two extractions employing a low-boiling-range fraction of petroleum ether. Much greater stability of chloroplasts resuspended in aqueous media has been attained by making up the phosphate buffer in 15 per cent methyl alcohol. Higher concentrations (0.001 M) of 2,6-dichlorophenol indophenol and opal-glass spectrophotometer cells kindly given to the laboratory by Dr. Kazuo Shibata were used. The photochemical reaction rates were calculated from the amount of dye reduced in the first half-minute illumination intervals. Table 3 presents typical data obtained during such β-carotene reactivation experiments.

The optimum concentration of β-carotene required for reactivation as indicated in table 3 is approximately 100 times greater than that used by Lynch and French.

Lyophilized chloroplasts prepared in later studies from both pokeweed and chard did not exhibit as great a reduction in Hill activity with petroleum ether as was observed in earlier trials. Such materials showed much greater reactivation with the extract than with β-carotene. It was observed that certain of the lyophilized preparations could be extracted continuously at room temperature for 18 hours or longer in a Soxhlet apparatus, and that the product retained as much as 50 per cent of the original activity, which could be further enhanced by adding back a portion of the extract. Petroleum ether fractions with elevated boiling range (50° to 70° C), however, caused greater irreversible loss in



activity than did fractions with a lower boiling range. The very marked stability of dry chloroplasts to petroleum ether treatment is in contrast to their great lability when suspended in water solutions.

Other petroleum ether-soluble compounds that exhibited the reactivation effect included  $\alpha$ -carotene and cryptoxanthine. Compounds without effect in terms of reactivating photolysis in petroleum ether-extracted chloroplasts at con-

TABLE 3. Effect of Petroleum Ether Extraction on Hill Activity in Lyophilized Poke-weed Chloroplasts, and of  $\beta$ -Carotene Re-activation

Treatment	Activity,* $\mu\text{M} \times 10^{-2}$ dye/ mg chlorophyll/min
None .....	242
Extracted twice with petroleum ether (b.p. 26–50° C) .....	7
Extract added back † .....	170
$\beta$ -Carotene added back in various concentrations, mg/mg chloroplasts	
1.5 .....	116
0.75 .....	112
0.44 .....	55
0.30 .....	35

\* Calculated on the basis of chlorophyll concentration of 8.4 per cent in the lyophilized chloroplasts.

† Extract from 8 mg chloroplasts added back to 4 mg extracted material used for photolysis measurement.

centrations similar to that required with  $\beta$ -carotene were menadione, corn oil, white mineral oil, paraffin, tocopherol, and  $\alpha$ -tocopherol acetate. The following compounds inhibited residual Hill activity in extracted chloroplasts: cholesterol, lecithin, ethyl oleate.

From these results it is believed that some fat-soluble factor or factors in addition to  $\beta$ -carotene are involved in the reactivation of petroleum ether-extracted chloroplasts. Future work will be devoted to the determination of the nature and mode of activity of these compounds.

# EFFECT OF LIGHT AND TEMPERATURE ON PIGMENT RATIOS IN BLUE-GREEN ALGAE

Per Halldal

Blue-green algae contain chlorophyll *a*, phycocyanin, phycoerythrin, and a number of different yellow carotenoid pigments. The color of the blue-green algae varies greatly under different growth conditions, owing to changes in the pigment ratios. Both the spectral composition and the intensity of the light have been assumed to be important in this respect. The first attempts to analyze in the laboratory the factors affecting these changes were made at the turn of the century. Among the factors affecting the color of algae are the age and nutritional status of the culture and the light to which it is exposed. From laboratory experiments and from the distribution of algae in nature two theories of the effect of light upon the pigment ratios in algae arose: (1) The color that the algae assume is due to a *complementary chromatic adaptation*, depending on the spectral composition of the light; e.g., at greater depth in clear water the red and the blue-green algae have a universal red color which is complementary to the blue light that penetrates to this depth. (2) The color is due to an *intensity adaptation* which is independent of the spectral composition of the light; e.g., the red and the blue-green algae at greater depth are shade plants which, at low light intensity, form a high amount of the accessory pigments phycocyanin and phycoerythrin, in order to catch a high percentage of the light. Today there is no general agreement as to which, if either, of these two theories is correct. It seems as though there is much to be said in favor of both.

In Year Book 55, page 261, an apparatus was described in which algae, grown on agar, were exposed simultaneously to crossed gradients of light intensity and temperature. In the course of the experiments it was evident that this method was well suited for the study of pigment for-

mation and destruction as influenced by light and by temperature.

When the blue-green alga *Anacystis nidulans* Drouet (Kratz and Allen's strain) was grown in the apparatus, the growth area was characterized by regions of very different colors. The change in color from one part of the growth area to another was apparently due to differences in both the absolute and the relative amounts of pigments present.

In order to study the pigments formed in different parts of the growth area, pieces of agar containing the algae were cut off the plate and placed upon a piece of opal glass. The absorption spectra of these samples were recorded with the Beckman DK-2 spectrophotometer. This procedure was suggested by Dr. Shibata during his stay at the Department last year. Many of the spectral curves obtained in this way had quite different shapes, owing to variations in the relative amounts of pigments present. Such curves should give opportunities to analyze *in vivo* the absorption characteristics of some of the pigments present in *Anacystis*. This analysis has been attempted by means of the graphical computer described in Year Book 52. The relative concentrations of the various pigments in the algae grown under different conditions were thus determined. Owing to great variations in the kinds as well as in the relative amount of yellow pigments, no single curve could be developed for them.

The existence of two different chlorophyll *a* forms *in vivo* has been suggested by several investigators. Only one form of chlorophyll *a* is known in solutions. The two different forms that seem to be present *in vivo* are therefore assumed to be due to different bindings of chlorophyll to other substances, presumably proteins.

Under certain assumptions, each absorption curve of *Anacystis* could be split up into curves for its components: two forms of chlorophyll *a*, phycocyanin, and yellow pigments. The last were obtained by subtraction of chlorophyll plus phycocyanin

curves from the total absorption. The relative amounts of these different pigments at various places in the growth area were calculated approximately and are represented in the three-dimensional diagrams of figure 11. In these diagrams the light-intensity scale and the temperature scale were on the horizontal plane, and the amount of pigments was plotted vertically on this field. By taking samples at different times during the experiments, time was also introduced as a variable.

These data showed that *Anacystis* went through an intermediate stage of development in which both chlorophyll and phycocyanin content were high over a wide range of temperature and light intensity, with the higher phycocyanin content at very high temperature near to the killing boundary and at high light intensity, around 600 to 800 foot-candles. The phycocyanin content lessened toward both lower light intensity and lower temperature. Maximum chlorophyll content was found at both intermediate temperatures (30° to 40° C) and intermediate light intensities (200 to 400 foot-candles). The content of yellow pigments was greatest around 400 to 800 foot-candles, and from 30° to 35° C. Thus, the greatest amount of yellow pigments was produced in the region between the maxima for chlorophyll and for phycocyanin. From this intermediate stage a growth pattern with striking differences in color developed on later days.

In the center of the growth area, from around 30° to 42° C, and from 500 to 1000 foot-candles, the color turned yellow. In a very narrow stripe at temperatures from 42° to 45° C and from 500 to 1000 foot-candles the color was bluish green. It was a purer green at lower light intensities over a wide range of temperatures. The absorption spectra showed that in the yellow region the amount of yellow pigments was very high, the chlorophyll content was moderate, but practically no phycocyanin remained. In the narrow stripe at high temperature the phycocyanin content had a maximum, and also the con-



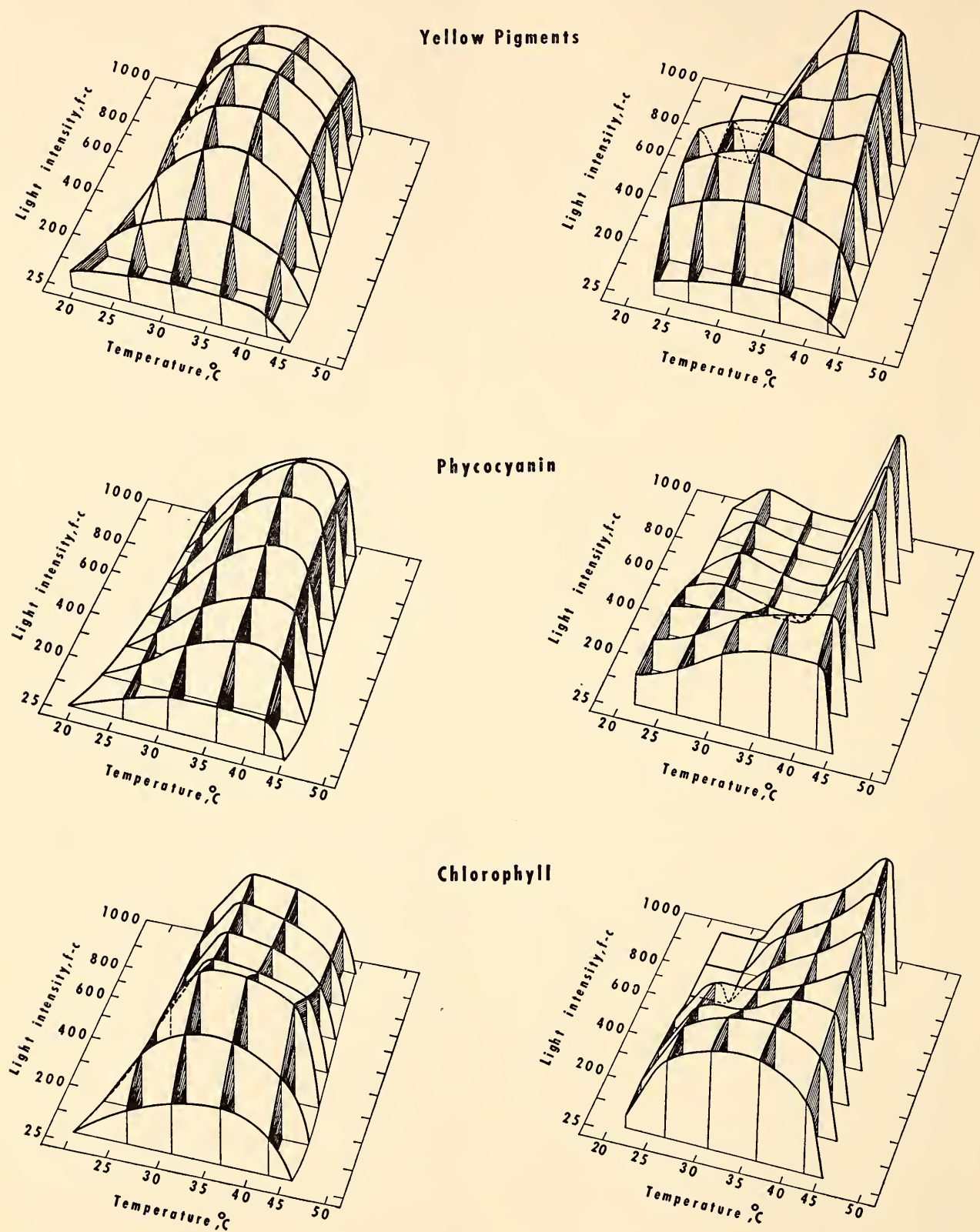


Fig. 11. The relative content of pigments in *Anacystis* after about 15 (left) and after 48 (right) hours' growth.

tent of chlorophyll was high in this area. Both chlorophyll content and phycocyanin content were high at low light intensity over a wide range of temperatures. If we may assume that great photosynthetic activity occurs where the content of chlorophyll and phycocyanin is high, these results suggest that high light intensity and intermediate to low temperatures are unfavorable for the photosynthetic apparatus in *Anacystis*. The remarkably narrow stripe at the extreme upper temperature limit for growth suggests that these conditions are favorable for photosynthesis in *Anacystis*. Yellow pigments formed at high light intensity possibly have a light-filtering effect that might protect the photosynthetic apparatus.

In the region where carotenoids are high in proportion to chlorophyll and phycocyanin there are two contradictory hypotheses to account for the observed results. One idea is that more carotenoids develop where needed as a light screen to protect the chlorophyll from bleaching by excess light. The contrary idea is that the chlorophyll has already been reduced to a lower level by the excess light that bleaches chlorophyll but not carotenoids. The present experiments do not distinguish between these two equally reasonable explanations.

One *Anabaena* species, obtained from Dr. M. B. Allen, did not develop markedly different growth zones like those of *Anacystis*. *Anabaena* grew more uniformly over a wider area. The growth boundary at high temperature was 44° C at 1000 foot-candles, and 45° C at 25 foot-candles. The boundary at lower temperature was 28° C at 100 foot-candles, and 25° C at 25 foot-candles. *Anabaena* showed, however, a gradual change in color from low light intensity to high. At low light intensity it was bluish green; at intermediate light intensity, yellowish green; and at high intensity, muddy green. When samples from this alga were analyzed in the spectrophotometer the relative amount of yellow pigments was found to be very high at high light intensity. Toward lower light intensity the relative amount of phyco-

cyanin increased. The accessory red pigment phycoerythrin was also formed in appreciable amounts at very low light intensity.

We have not analyzed the effect of light of different spectral regions on the color of algae, but the above results show that the intensity of the light is a very important factor in determining the color of the algae. The experiments with *Anacystis* show that temperature also is important, and that a striking interaction exists between these two factors.

The results from the *Anabaena* experiments suggest that two types of adaptation took place: at low light intensity, accessory pigments such as phycocyanin and phycoerythrin were formed in order to achieve a higher photosynthetic capacity; at high light intensity, the amount of these pigments was reduced and yellow pigments, possibly acting as light screens in order to protect the photosynthetic apparatus, were formed.

A requirement for successful culture of blue-green algae in the laboratory is very low light intensity. In nature these algae are often exposed to direct sunlight of the highest possible intensity. Under these conditions they are, as a rule, dark brown to black. It may be that the conditions in nature favor the formation of some photosynthetically inactive carotenoid pigments at high light intensity which act as a protective screen.

#### GROWTH PATTERNS OF VARIOUS ALGAE IN CROSSED GRADIENTS OF LIGHT INTENSITY AND TEMPERATURES

Per Halldal, Helen S. Huang, and C. S. French

Work on the interrelated effects of light intensity and temperature on the growth of various species of algae is being continued. The algae are grown on an agar surface having a gradient of temperature left to right and a gradient of light intensity front to back. The growth pattern produced on such a plate has been found to be very different in different species of algae.



These experiments were started to develop a method of screening algal strains for their suitability to large-scale culture,

temperatures is described in another section. In this section only the growth patterns illustrated in figure 12 will be dis-

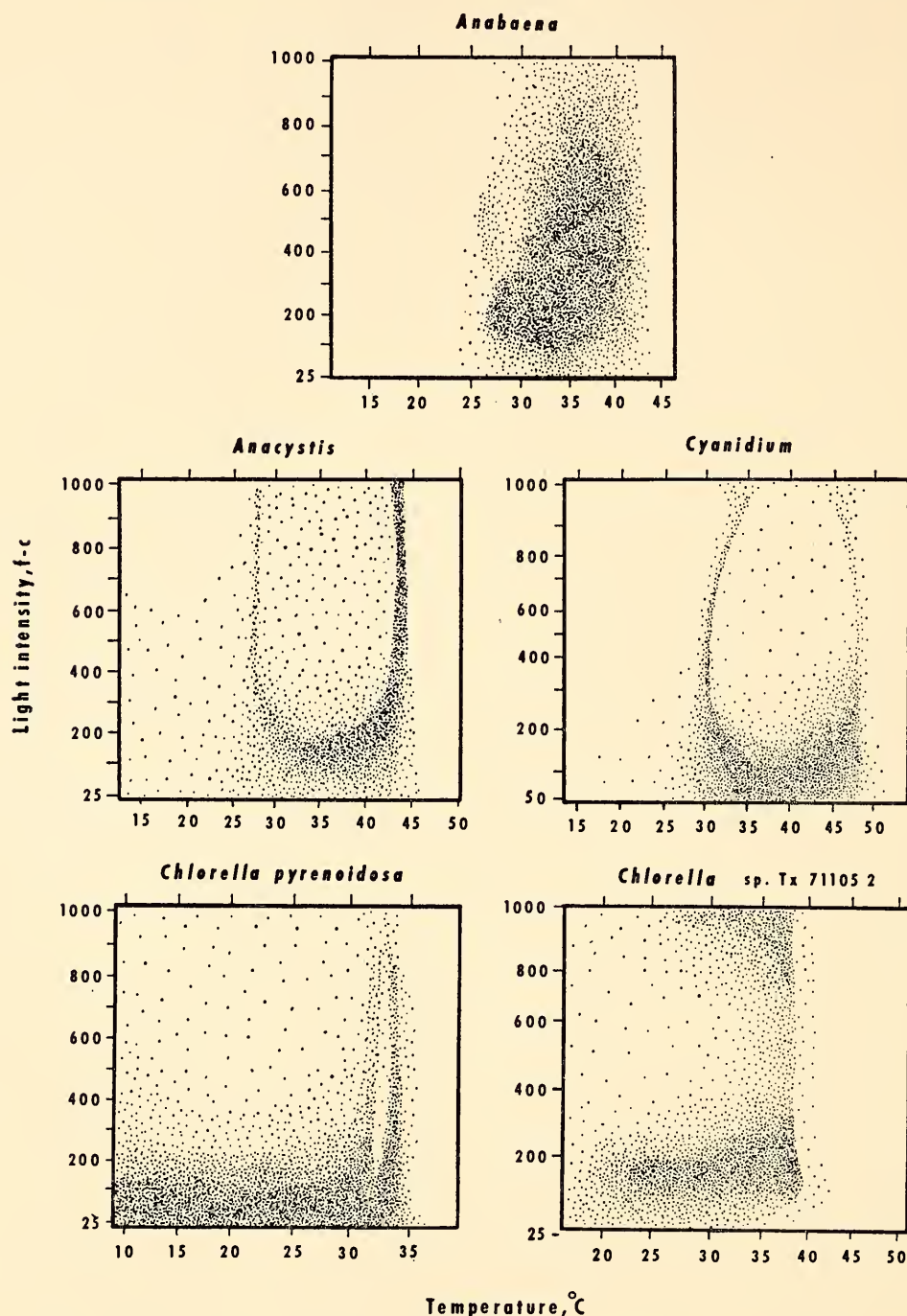


Fig. 12. The influence of temperature and light intensity on the growth of several algae.  
*Anabaena* sp., from Dr. M. B. Allen, 4-day culture.  
*Anacystis nidulans*, from Dr. Jack Myers, 2-day culture.  
*Cyanidium caldarium*, from Dr. M. B. Allen, 3½-day culture.  
*Chlorella pyrenoidosa*, Emerson strain, from Dr. Jack Myers, 6-day culture.  
*Chlorella* sp., Tx 71105-2, from Dr. Jack Myers, 2-day culture.

for which it may be necessary to have various strains for different localities or different times of the year. The cross-gradient procedure for studying pigment formation under a series of controlled intensities and

cussed. The growth pattern produced on this plate must bear some relation to the natural habitat of different algal species.

It has been known for some time from the work of Myers that the rate of photo-

synthesis of algal cultures may for short times considerably exceed their long-time growth rate under the same conditions. Growth, while dependent on photosynthesis for the supply of food, is a considerably more complex process. Many different and poorly understood steps come between photosynthesis and the production of algal cells. It should therefore not be surprising to find that the simple variables of light intensity and temperature may influence growth differently from the way they influence photosynthesis.

The plate described in Year Book 55, pages 261-265, was uniformly inoculated for each experiment with a liquid culture of the alga. The algae were allowed to settle and the excess liquid to evaporate before the temperature and light-intensity gradients were established. The plates were photographed at intervals, and samples were taken from some for spectroscopic observation of the pigments produced at different places. No measurements were made of actual cellular material produced, so that "growth," as here used, refers only to the apparent cell density as evidenced by depth of color.

In last year's report the L-shaped growth pattern of *Chlorella pyrenoidosa* was mentioned. It has been found recently that the vertical arm of this L-shaped pattern is accentuated by a very narrow temperature range around 32° C in which growth is poor at high light intensities. On either side of this temperature range, of about 1°, the growth is better. On the higher-temperature side of this sharply defined range of poor growth is an equally narrow range of luxuriant growth which forms the vertical arm of the L. At temperatures below 32° C at high light intensity the growth is moderately profuse for a few degrees, then weakens gradually toward lower temperatures.

At intensities of light lower than about 150 foot-candles the growth of this strain of *Chlorella pyrenoidosa* is almost independent of temperature within the limits of about 12° to 34° C. Thus, at low intensities, growth is more or less what it

would be expected to be on the basis of the known behavior of photosynthesis in this species. The rate of photosynthesis is more or less independent of temperature at low intensity where light is the limiting factor. The decrease in the growth rate at higher intensities in the middle temperature range is presumably due to the solarization effects of high light intensity. No obvious explanation appears to account for the sharp drop-off in growth rate at 32° C at high light intensities, or for the high growth rate in a 1° C region just above this temperature of poor growth.

Comparison of the *Chlorella pyrenoidosa* patterns with those for the high-temperature *Chlorella* Tx 71105-2 brings out many points of interest. First, it is evident that the growth range extends to higher temperature limits, as has been well established for this strain by liquid culture experiments. Second, its growth at very low light intensity is relatively poorer than for *Chlorella pyrenoidosa*. Third, a narrow region of thin growth within which yellow cells are produced occurs at high temperatures and all intensities near the upper temperature survival limit. (An apparent increase of growth at high intensity over that at intermediate intensity shows on the diagram but is believed to be due to an inhomogeneity of the inoculum or of the light field in that particular experiment.)

The blue-green algae *Anabaena*, *Anacystis*, and *Cyanidium* differ from *Chlorella* strains in having their cutoff limit of growth at a higher temperature. *Anabaena* showed the least complicated pattern of these three. Its growth is almost independent of intensity from about 50 to 900 foot-candles. Temperatures from 27° to 42° C support a reasonable amount of growth within these intensity limits, though at intensities from 250 to 1000 foot-candles there is a stripe of yellowish brown cells extending diagonally from 25° to 38° C. The temperature range of growth is slightly broader at low intensity, and no narrow regions of temperature tolerance were observed. *Anabaena* cannot be suspended uniformly, so that the resulting pictures



are not so good as those of the single-celled algae for showing details of the growth pattern. The growth in small clumps may have reduced the actual intensity available to the underlying cells. *Anabaena* does, however, appear to tolerate high intensities better than any of the other algae we have tested, even including the Texas high-temperature *Chlorella*.

The unicellular blue-green *Anacystis* showed a most remarkable L-shaped pattern having a region of intense growth at  $44 \pm 1^\circ \text{C}$  at high light intensity, extending at lower intensities down to about  $30^\circ \text{C}$ . This sharp line of high growth rate at high intensity was not complicated by an adjacent narrow range of low growth rate like the one found for *Chlorella pyrenoidosa*. At temperatures just below  $44^\circ \text{C}$ , the growth was uniformly weak over about a  $10^\circ \text{C}$  temperature range where the cells turned yellow. An increase of growth of green cells was observed just before the low-temperature cutoff at about  $30^\circ \text{C}$ . By contrast with *Chlorella*, *Anacystis* showed very little extension of the low-temperature growth limit at low intensity. In the high-temperature range above which no growth took place, all the algae bleached out completely. *Anacystis* bleached at low temperature and high intensity; *Anabaena* and *Cyanidium* bleached at low temperature and all intensities.

In the patterns of all the algae discussed so far there is at least a slight extension of the temperature tolerance range at low intensities. With *Cyanidium*, however, the opposite effect was observed. In this barrel-shaped pattern the growth at low intensities and at high intensities occurred over a narrower temperature limit than in the intermediate intensity range. Thus the temperature cutoff limits are narrower at low than at intermediate intensity. The *Cyanidium* pattern showed a green streak at the limit of growth at both high and low intensities. The area between them had a yellowish brown color and somewhat resembled the poor-growth streak on the *Chlorella* plate but had a very much

wider temperature range. At low intensity the algae were green from  $30^\circ$  to  $47^\circ \text{C}$ .

In *Cyanidium* there was a small increase in growth rate at about  $42^\circ$  to  $47^\circ \text{C}$  in the 100 to 250 foot-candle range above that at other places on the plate. A region of accelerated growth rate was also found along a curve from about 200 foot-candles at  $33^\circ$  to about 75 foot-candles at  $45^\circ$ .

The strikingly narrow temperature range of dark green growth of cultures that have grown for several days is believed to be caused by a combination of factors. In comparing pictures taken at successive time intervals it is evident that the maximum growth of the young cultures occurs at somewhat lower temperatures than that of the final dark green stripe. This earlier growth presumably exhausts the nutrients, probably the nitrogen supply, in a limited region on the plate, thus leading to the formation of yellow cells as previously described by Spoehr and Milner. The dark green region therefore moves to higher temperatures where previous growth had been limited by the excessive temperature. As the region of nutrient exhaustion approaches the upper temperature limit for growth the green region becomes sharply defined and very narrow.

The older patterns of growth on the plate as shown in figure 12 are very characteristic for different algae, but the first detectable pattern is to be preferred for the selection of the intensity and temperature for optimum growth rates.

#### PHOTOTAXIS IN MOTILE ALGAE

*Per Halldal*

The orientation of freely moving organisms according to light direction is termed topophototaxis: a swimming toward the light is designated positive topophototaxis; away from the light, negative topophototaxis. A similar light response occurs in motile algae. An organism that will show positive phototaxis under certain condi-

tions may show negative phototaxis under other conditions.

The reversal from positive to negative topophototaxis, and vice versa, has been claimed by different investigators to be caused by several factors. Most textbooks in botany say that a positive reaction occurs at low light intensity and is reversed at very high light intensity, but other factors such as the oxygen and carbon dioxide pressure in the water have also been assumed to influence the movement of algae directly. During the past year we have analyzed the reversal from positive to negative reaction, and vice versa, in the unicellular green alga *Platymonas subcordiformis* (Wille) Hazen (Gibor's strain). We have found that, when the alga showed a certain response, the reaction could not be reversed immediately by altering either the light intensity or the absolute or partial pressure of  $\text{CO}_2$  or  $\text{O}_2$  in the medium. Light intensity,  $\text{CO}_2$ , and  $\text{O}_2$ , therefore, cannot be primarily responsible for the change.

On the other hand, we have found that  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  are directly involved, and that they are antagonistic in their effect.  $\text{Ca}^{++}$  causes a negative and  $\text{Mg}^{++}$  a positive phototaxis. A number of other ions tested produced no reaction.

The effect of  $\text{Ca}^{++}$  was measured on the negative phototactic activity, recorded as swimming rate, obtained with different concentrations of  $\text{CaCl}_2$  in a  $\text{CaCl}_2 + \text{NaCl}$  solution adjusted to ionic strength  $1.0 \mu$ ; and the effect of  $\text{Mg}^{++}$ , on the positive phototactic activities, recorded as swimming rate, with different concentrations of  $\text{MgCl}_2$  in a  $\text{MgCl}_2 + \text{NaCl}$  solution. For  $\text{MgCl}_2 + \text{NaCl}$ , the ionic strength was adjusted to  $1.0 \mu$  at  $\text{MgCl}_2$  concentrations below  $0.33 M$  ( $1.0 \mu$ ). Above this concentration the ionic strength of  $\text{MgCl}_2$  was higher than  $1.0 \mu$ , and  $\text{NaCl}$  was therefore omitted.

The algal cells were motionless in concentrations of  $\text{CaCl}_2$  below  $0.0045 M$ . They showed maximum activity around  $0.06$  to  $0.09 M$ , and were motionless at concentra-

tions above  $0.3 M$ . In the  $\text{MgCl}_2$  solution the cells were motionless below  $0.01 M$ . With increasing concentration, the positive activity rose to a maximum around  $0.15 M$ , then decreased very slowly with increasing  $\text{MgCl}_2$ . No motion was observed at concentrations above  $1.75 M$ .

If  $\text{CaCl}_2$  and  $\text{MgCl}_2$  were mixed in proper amounts, and the ionic strength and the hydrogen-ion concentration were within certain limits (ionic strength  $0.65$  to  $1.15 \mu$  adjusted with  $\text{NaCl}$ ,  $\text{pH}$   $5.5$  to  $8.5$  with buffer solutions), a random-motion stage could be obtained in *Platymonas*. At  $\text{pH}$   $6.0$  this stage was obtained when the molar ratio  $\text{CaCl}_2:\text{MgCl}_2$  was around  $1:6$ . If at this  $\text{pH}$  the ratio was greater than  $1:6$  a negative phototaxis occurred; if it was less, a positive one.

The theory that the driving power in the flagellates is an "adenosine triphosphate (ATP) motor" has recently been suggested by Links in Leyden, Holland. If this is so, it means that the energy supply to muscle fibrils in animals and to the flagellar apparatus in algae is the same. It is known from muscle research that  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  may be involved with antagonistic effects in the ATPase activity. The antagonistic effect of  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  in phototaxis suggests a related mechanism in the flagellates.

#### AN INQUIRY INTO THE CAUSES OF ALBINISM

James H. C. Smith, Lois J. Durham, and  
Charles F. Wurster

Koski and Smith established that certain albino mutant corn leaves produce protochlorophyll in the light just as effectively as normal corn leaves. The reason these leaves fail to green in continued illumination is that the chlorophyll formed is bleached by the light. The recent examination of several other albino corn mutants, generously supplied to us by Professor E. G. Anderson, of the California Institute of Technology, and Professor Donald Robertson, of Iowa State College, has completely supported these earlier observations.

Up to the present time the causes of



bleaching in these mutants have not been known. The possibility exists that the path of chlorophyll biosynthesis and stabilization may be genetically blocked at certain stages so as to permit the accumulation of photolabile pigments. Some of the possible consequences of this hypothesis have been investigated during the past year.

Granick obtained a *Chlorella* mutant with properties similar to those of the albino corn mutants. He found, however, that the chlorophyllous pigments were not esterified with the phytyl group as in normal plants. He suggested that albinism may be due to a block at the phytylating step in the biosynthesis of chlorophyll which produces the reputedly photolabile chlorophyllide instead of the photostable chlorophyll.

That the lack of the phytyl group in chlorophyll might be the cause of albinism was supported by the observation of Loeffler (cf. Year Book 54, pp. 159-160), who found that a considerable portion of the protochlorophyll-like pigments in dark-grown leaves and the chlorophyll-like pigments immediately derived therefrom by illumination lacked the phytyl group. These facts suggested that perhaps the pigments of albino leaves were phytyl-free and therefore unstable in light.

Our analysis of the pigments from the available etiolated corn mutants showed them to contain about the same fraction of their pigments in the phytylated and non-phytylated forms as normal corn leaves. This was true before as well as after they had been illuminated. Consequently, the lack of phytyl cannot be the essential cause of albinism.

Loeffler found, furthermore, that, when briefly illuminated etiolated corn leaves were placed in the dark, they quickly phytylated their chlorophyllide *a*—their acid pigment component—and thereby formed chlorophyll *a*. Most of the albinos either lacked this ability or had it very poorly developed. One albino mutant, however, was just as efficient in phytylating its chlorophyllide *a* as normal plants

and yet bleached as readily as the other albinos. From this observation, it is certain that lack of phytylating ability is not the sole cause of albinism.

Another possible cause of albinism is the inability of the albino mutants to stabilize their new-formed chlorophyll. In his measurements of the absorption spectra of intact, etiolated normal leaves made before and after they had been illuminated, Shibata noted that the chlorophyll first formed had an absorption maximum at 684 m $\mu$  (Year Book 55, pp. 252-256). After the leaves had stood in the dark for 20 minutes after their illumination, the maximum of absorption had shifted to 673 m $\mu$ . Further illumination of such leaves increased their chlorophyll content.

Since continued illumination bleached the chlorophyll of albino leaves, it was surmised that this shift in absorption might be evidence of a stabilizing action on the chlorophyll of normal leaves. If so, albino leaves should not exhibit the shift.

A spectroscopic examination of the albinos demonstrated that, although several of them showed little or no shift in the dark, at least one gave as pronounced a shift as normal leaves. Continued illumination of this mutant, however, always resulted in bleaching. From this behavior it is clear that the shift in itself is not correlated with chlorophyll stability.

The ability to shift the red peak is, however, roughly parallel to the phytylating capacity of the various corn mutants. The magnitude of the shift was found to correlate in some degree with phytylating capacity. Normal plants, and the one mutant, that have the greatest capacity for phytylation exhibit the largest spectral shifts of the new-formed chlorophyll; those mutants that have little or no ability for phytylation exhibit much smaller spectral shifts. The degree of correlation is by no means high, and from the quantitative aspects of the phytylation and the spectral shift it seems probable that the relation between the two is coincidental rather than causal.

In the analysis of the pigments in etio-

lated leaves before and after being illuminated, it was found that almost invariably the percentage of phytylated pigment was higher and of nonphytylated pigment lower in the original sample than in the sample immediately after illumination. From this finding it appears that light causes some photolysis of the ester. The consequence is not evident.

One further significant observation is that the albino mutant which most readily esterified the chlorophyllide was essentially carotenoid-free. It has been repeatedly suggested that the phytol group of chlorophyll is derived from the carotenoids. The facts presented here point to another source of the phytol than the carotenoids, perhaps a blocked carotenoid biosynthesis.

#### ETIOLATED LEAVES OF LARGE SIZE

*James H. C. Smith, Charles F. Wurster, and Malcolm A. Nobs*

Almost all leaves grown in the dark are small. Since their smallness frequently makes certain types of measurements on them difficult to perform, we have tried for a number of years to find a plant or a technique that would produce leaves in the dark with a large area of mesophyll tissue. Our previous attempts have been unsuccessful.

Recently, however, a plant has been found that produces a relatively large leaf in the dark. It is *Colocasia esculenta* Schott, commonly known as taro. Tubers obtained in the market were planted in sand and grown in the dark at about 25° C. After 3 weeks, the leaves were ready for harvesting. They had areas of roughly 50 sq cm, and a weight of 10 to 12 grams.

A part of a leaf was placed in the opal-glass apparatus (p. 282), and its absorption was measured before illumination, and afterward at different intervals in the dark. Before illumination, the leaf contained protochlorophyll holochrome with an absorption maximum at 648 mμ. Immediately after illumination, it contained chlorophyll holochrome with an absorption

maximum at 685 mμ, and, after standing in the dark for 30 minutes, it had an absorption maximum at 669 mμ—an unusually large shift for the chlorophyll-holochrome absorption peak. During the 30-minute sojourn in the dark, no new protochlorophyll was formed, as was evidenced by the absence of increased absorption at 648 mμ, nor was additional chlorophyll formed by a subsequent 30-minute exposure to light. On standing in room light, however, the leaf slowly greened. Thus, this leaf behaved like other normal leaves except in the rapidity of new protochlorophyll and chlorophyll formation. Leaves of this plant appear to be well suited for experiments in which a relatively large area of etiolated leaf surface is desirable.

#### DERIVATIVE SPECTROPHOTOMETRY

*C. S. French and Gordon E. Harper*

In Year Book 55, progress on the construction of a derivative spectrophotometer was reported. The general operating principles mentioned last year and stated more explicitly below are still followed, but the apparatus has been greatly modified to improve the performance. Last year the instrument was suitable for point-by-point measurements but would not perform adequately when sweeping through the spectrum automatically. Since then, the measuring amplifiers have been rebuilt and a second servo system has been added to control the signal level during a measurement; the machine is now in use.

The measurement of first derivative of absorbance with respect to wavelength is based on the following principles. Absorbance, or optical density, is defined as  $E = \log (1/T)$ , where  $T$  is the fractional transmission of the sample. Differentiating,  $dE = -dT/T$ , or, for a constant value of  $\Delta\lambda$ ,  $\Delta E/\Delta\lambda = K(\Delta T/T)$ . Voltages proportional to  $I \Delta T$  and to  $IT$  ( $I$  being the effective light intensity) are produced by an optical system and a photomultiplier operating as in figure 13. The ratio of the



$I \Delta T$  and the  $IT$  voltages  $\Delta E$  is plotted by a recorder against wavelength,  $\Delta \lambda$  being a constant.

The light beam continuously varies over a small wavelength interval,  $\Delta \lambda$ , at 100 cps. The beam alternately goes through the reference and the sample cell at 8 cps. The beam through the sample cell is interrupted by a 600-cps chopper. The electrical signals generated in the photomultiplier by these beams are alternately sent to appropriate amplifiers by switches synchronized with the mirror that sends the light beam either to the reference or to the sample cell.

stant wavelength interval,  $\Delta \lambda$ , is swept through the spectrum of a grating monochromator. The block diagram, figure 14, shows how the optical and electronic components are arranged to put these principles into practice.

A calibration device is mounted so that it can be rapidly inserted in the sample beam at an image of the small part of the spectrum,  $\Delta \lambda$ , over which the slit vibrates. The calibrator is made of six uniformly spaced pins tapered to give a light attenuation in the spectral plane of  $\Delta E = 0.005$  optical density unit per millimicron.

Tests with glass filters have shown that

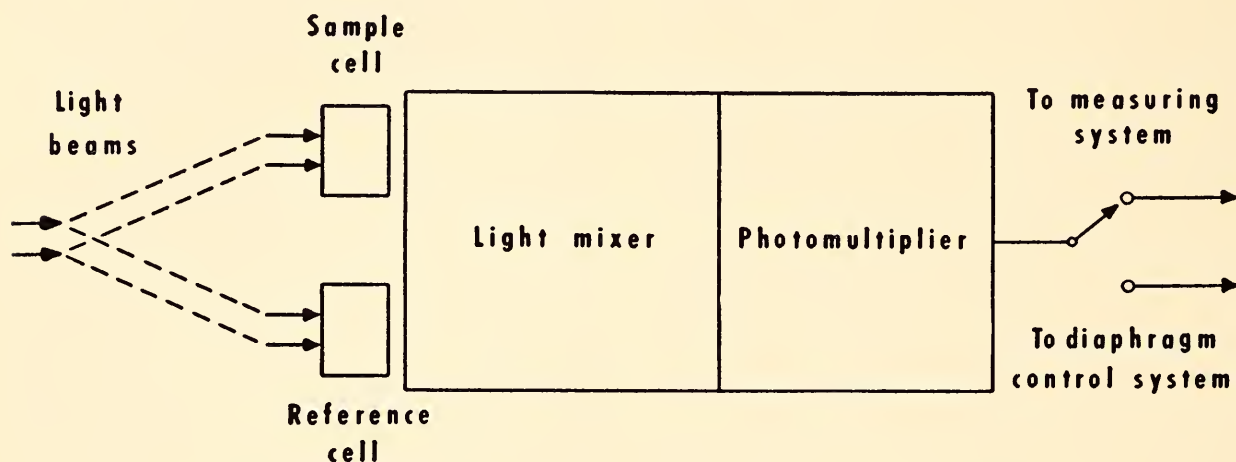


Fig. 13. Part of the optical arrangement of the derivative spectrophotometer.

While the light goes through the reference cell the 100-cps signal operates a motor-driven diaphragm to equalize the effective intensity over the  $\Delta \lambda$  interval. Thus, when the beam goes into the sample cell, its intensity is constant over  $\Delta \lambda$ . Therefore, any 100-cps modulation of the beam coming out of the sample must be due to a difference in transmission,  $\Delta T$ , of the sample over the wavelength interval,  $\Delta \lambda$ . From the sample beam we have two voltages:  $V_{100} = KI \Delta T$ , and  $V_{600} = K'IT$ . These voltages, differing in frequency, are separated by tuned amplifiers, rectified, and filtered. Since

$$\frac{V_{100}}{V_{600}} = \frac{KI \Delta T}{K'IT} = K'' \frac{\Delta T}{T} = K''' \Delta E$$

their ratio  $\Delta E$  is continuously plotted by a recorder against wavelength as the con-

differences in the optical density of less than 0.0005 over a 1.5-m $\mu$  interval can be resolved. Records of derivative spectra of chlorophyll in ether solution and in live cells are given in another section of the report. Though further tests and improvements may be necessary, the apparatus is now being used in studies of chlorophyll spectra in photosynthetic organisms.

#### A SPECTROPHOTOMETER ACCESSORY FOR MEASURING ABSORPTION OF TRANSLUCENT SHEETLIKE SAMPLES

James H. C. Smith and R. W. Hart

The opal-glass technique developed by Shibata (cf. Year Book 55, pp. 252-256) for measuring absorption spectra of colored translucent materials has proved its worth for following pigment changes in intact pieces of etiolated leaves during the past

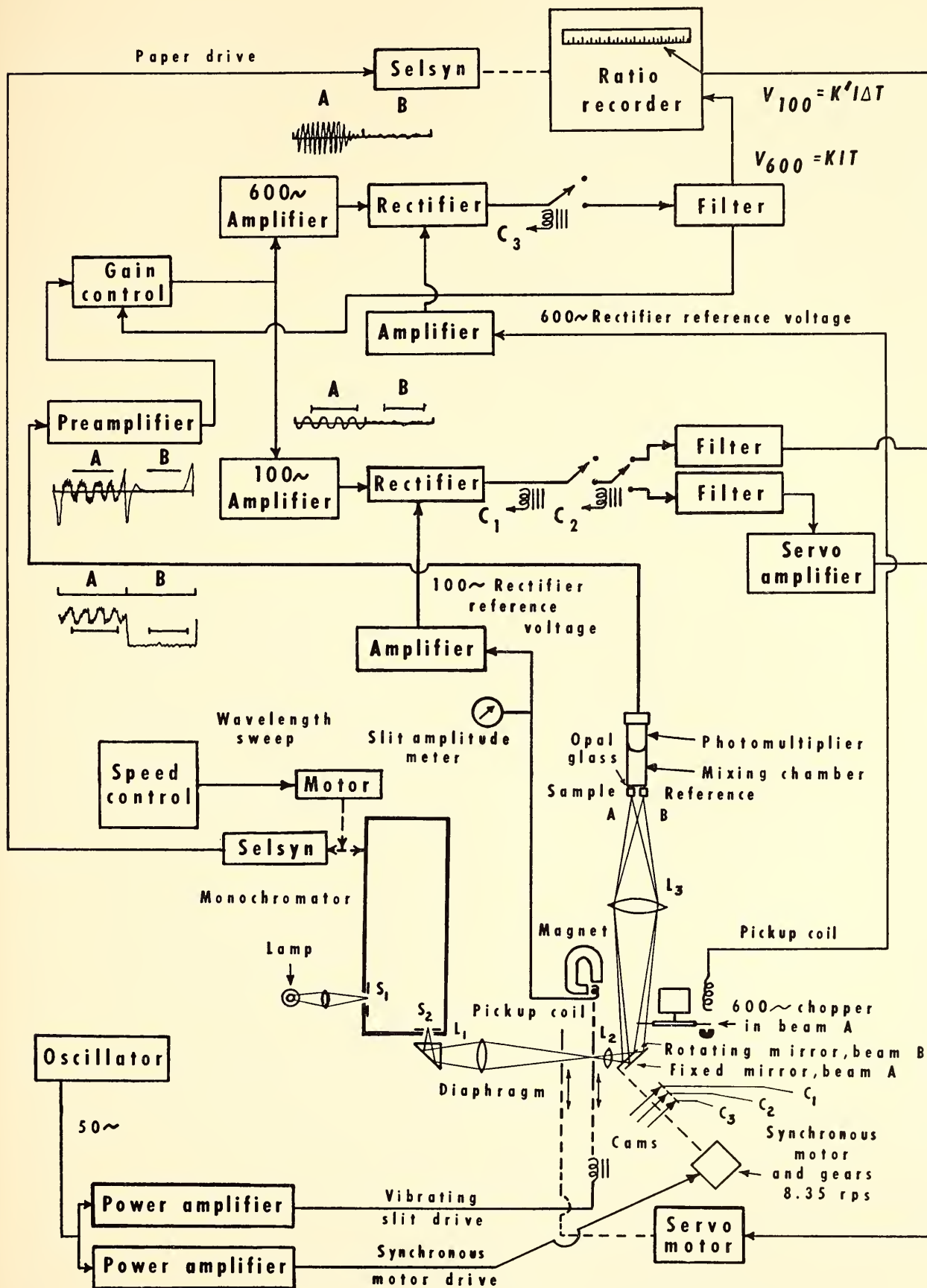


Fig. 14. A block diagram of the derivative spectrophotometer.



year. We have constructed an accessory to the Beckman DK-2 recording spectrophotometer which is far more convenient for routine use than the improvised setups. The new device is well suited to making routine observations on leaves and other translucent films and laminae.

This accessory substitutes for the regular cell holder of the Beckman instrument. It consists of a sample holder and a light-intensity attenuation device mounted on a base that fits snugly the two positioning pins of the cell-holder compartment of the spectrophotometer.

The sample holder is made of two black

screws in the cross bar. With the sample in place, and the spectrophotometer set in a region where the sample does not absorb, the two spectrophotometer beams are equalized by adjusting the screws. After this, the absorption spectrum of the sample is measured in the usual way.

The device has been employed to measure the absorption spectra of a large number of intact etiolated normal and albino leaves and to follow the spectral changes induced in them by different treatments after their illumination. The results have been treated in another section of the report.

TABLE 3. Light Absorption by Chlorophylls in Solution and Adsorbed on Filter Paper

State	Chlorophyll <i>a</i>			Chlorophyll <i>b</i>		
Adsorbed						
Absorption maxima, mμ.....	668	624	435	651	602	468
Relative optical densities.....	3.25	1.00	4.10	2.93	1.00	6.55
Dissolved in ether						
Absorption maxima, mμ.....	660	614	429	641.5	592	454
Relative optical densities.....	6.00	1.00	7.78	4.40	1.00	13.1

metal plates in contact with each other normal to the light beam. The back plate carries two opal-glass windows; the front plate has two clear-glass windows. The sample, usually a piece of leaf, is held in the measuring beam between one pair of windows, and the control intercepts the beam passing through the other. The plates, held together by springs, keep the leaf or any other sheetlike substance between them. Through a tongue-and-groove arrangement, the sample holder can be reproducibly seated on the holder base. The sample holder is as near the back wall of the cell-holder compartment of the spectrophotometer as possible.

The light-intensity control device is a T-shaped metal piece that carries two large

A further use of the apparatus has been to measure the absorption spectra of pigments adsorbed on filter paper, such as the "spots" obtained by paper chromatography. (Cf. Loeffler, Year Book 54, pp. 159-160.) Even though the absorption spectra of the adsorbed pigments may differ from those of the dissolved pigments, they have characteristic shapes that can be used for identification, especially if the curves for the adsorbed pure pigments are known.

The absorption spectra of the adsorbed pigments lie at longer wavelengths than those of the dissolved pigments, and the higher absorption peaks are "flattened" relative to the lower peaks. Examples of these effects are given in table 3.

EXPERIMENTAL TAXONOMY

With the completion of Volume IV of *Experimental Studies on the Nature of Species*, which deals with the genetic structure of climatic races in relation to environ-

ment, the emphasis of the work of the group in Experimental Taxonomy is being shifted from investigations utilizing primarily cytological, genetic, and transplant

techniques to studies on the comparative physiology of ecologically diverse but closely related taxonomic entities.

This newer approach follows logically from the investigations that have been completed. Many of the problems about general relationships between and within species that seemed so confusing twenty-five years ago have been clarified. The methods and viewpoints that have been developed are now available to investigators everywhere. Undoubtedly they will be applied sooner or later to many of the thousands of groups of plant species that still remain to be studied experimentally.

Further penetration into an understanding of basic principles of plant relationship appears to be dependent upon the development of methods for the study of mechanisms underlying natural selection. The general nature of the products of natural selection is now fairly well known; in contrast, very little is understood about even some of the most elementary questions that pertain to the internal functioning of plants as influenced by their hereditary composition and by their environment. The comparative study of the physiology of taxonomically related forms differing in hereditary composition thus becomes a field of first importance for critical investigation.

During the current year some progress has been made in this direction in the study of photosynthesis and respiration of altitudinal races of the *Mimulus cardinalis* complex. Concurrently with these physiological studies, a genetic analysis of the inheritance of both morphological and physiological characters in the same group of plants seems to offer essential background information for interpreting the physiological data.

A comprehensive study of the taxonomy, ecology, and comparative physiology of various races, species, and genera of the Lemnaceae, a family of small floating aquatic plants, has been summarized for publication by Elias Landolt; it marks an important beginning in the study of com-

parative physiology of closely related taxonomic forms. This work, initiated by Dr. Landolt while on a Carnegie Institution fellowship with the Department, includes critical comparisons of growth rates of species and races collected in many different environments. More than a hundred strains were isolated in pure culture for the purpose, and observations were made periodically at the original collection sites during different seasons to ascertain their behavior in the wild. Striking differences were observed in growth rates under controlled conditions between different genera and species, and sometimes between races of the same species. The relation between observed responses under laboratory trials and the characteristics of the various forms under field conditions is not always clear, but Landolt's study has laid the essential groundwork for further investigation of this unique group of plants, which is ideally suited for physiological techniques. His cultures, many collected in collaboration with members of the regular staff, are being maintained in the Department for further studies.

Dr. Ursula Brodfuehrer, formerly of the University of Munich, used the facilities at the Timberline Station during the summer of 1956 in connection with her laboratory studies at the Earhart Laboratory at Pasadena on the effects of ultraviolet radiation on the growth of *Arabidopsis*, *Mimulus*, and several alpine species native near Timberline. Her findings support earlier indications that exposure to ultraviolet radiation may influence growth, and that its effect is influenced by temperature. Plants growing in a temperature higher than their usual optimum show stimulation with small dosages of ultraviolet radiation.

The successful hybridization of widely distinct diploid species of *Achillea* native to central Europe, and the study of their progeny, throw new light on the evolution and relationships of complex aggregations of polyploid forms which are distributed throughout the northern hemisphere in a



wide range of climates. The new data, together with data obtained earlier in studies on altitudinal and latitudinal climatic races of North American tetraploid *A. lanulosa*, hexaploid *A. borealis*, and crosses between North American and European polyploid species, appear to make possible an approximate reconstruction of the evolutionary development of this entire complex.

New data on the performance of parental and hybrid bluegrasses from the widespread regional screening tests conducted by Agricultural Research Service, in addition to further information from plantings at the Soil Conservation Service nurseries, from collaborators in northern Europe, and from the altitudinal transplant stations, point to a general consistency in the performance of hybrid derivatives from given combinations of species. The range of recombinations that can be obtained from a particular cross, although wide, does not ordinarily transcend the limits set by the parents. Within broad limits, therefore, predictions can be made about the kinds of hybrid derivatives to be expected from crosses even in such a highly polyploid apomictic complex as *Poa*, in which an extensive reshuffling within the genomes contributed by each parent might have been looked for.

The possibilities and limitations for synthesizing new hybrid combinations of bluegrasses suitable for use in widely ranging climates now appear to be reasonably well established. The extensive studies on *Poa* are approaching a degree of maturity that will render possible the preparation of a critical summary for publication.

#### PHYSIOLOGICAL INVESTIGATIONS

William M. Hiesey, Harold W. Milner,  
and Malcolm A. Nobs

The general plan of the program on the comparative physiology of ecologically distinct races and species is to relate observations at the three altitudinal field stations with critical laboratory measurements of

physiological processes under controlled conditions. The greenhouse and nursery facilities at Stanford are, accordingly, being adapted for the growing of relatively fewer plants with increasing attention to factors of the external environment affecting their growth and development. The focus of the investigations will be laboratory experiments on basic physiological processes, at present on respiration and photosynthesis.

Concurrently with the physiological investigations, genetic experiments on the mode of inheritance of differences between the diverse ecological races of the same plant groups are to be carried on to furnish a supply of experimental plants having known cytological and genetic characteristics. In this manner the interaction between factors both of heredity and of environment as expressed in terms of physiological functions may be investigated. The teamwork approach that has proved effective in previous investigations will be followed.

Some progress in the general plan outlined above has been made during the current year, utilizing as plant materials members of the *Mimulus cardinalis* complex. As reported last year (Year Book 55, pp. 241-242), Dr. F. J. F. Fisher was successful in growing forms of lowland *M. cardinalis* and alpine *M. lewisii* from seed in bacteria-free cultures in Erlenmeyer flasks. Measurements of rates of photosynthesis and respiration were made by the method developed by Dr. John P. Decker, now with the U. S. Forest Service at Tempe, Arizona. These measurements confirmed the practicality of the infrared analyzer for measuring changes in CO<sub>2</sub> concentration in the flask cultures.

Later Dr. Decker brought his instrument from Tempe to Stanford and, in co-operation with members of the staff during a visit of three weeks, conducted exploratory studies on cloned plants of *Mimulus* grown in the greenhouse. Some of his results are summarized in later paragraphs.

Dr. Decker generously lent his instrument for trial use by the staff. Replicate determinations of photosynthesis of a given plant propagule agreed within 1 per cent. Determinations on vegetative propagules of individuals of *Mimulus cardinalis* grown under similar greenhouse conditions are being made, and the extent and cause of intraclonal variation are being studied. The measurements include rates of respiration in darkness at different controlled temperatures, rates of photosynthesis at various light intensities and temperatures, and compensation points (the level at which CO<sub>2</sub> concentration is maintained in balance by respiration and photosynthesis at a given temperature at a saturating light intensity). Differences of compensation points under given conditions may be a useful index of physiological behavior in comparing ecological races. The reproducibility of measurements on separate propagules of the same individual must be known before the studies can be extended to a comparison of individuals of different altitudinal races.

The substitution of an infrared analyzer for the Thomas analyzer in the apparatus described in Year Book 55, pages 239–241, is being considered; it would greatly speed the determination and simplify the calculation of the quantities of CO<sub>2</sub> essential to measurements of photosynthesis and respiration. For exploratory work using the infrared analyzer, a chamber with simplified controls was developed, in which temperature variation is held within  $\pm 0.2^\circ$  C, precise enough for initial studies, and the light intensity is changed by inserting screens of known transmissions between an incandescent flood lamp and the water filter that removes infrared radiation.

Besides the *Mimulus cardinalis* complex, altitudinal races of the *M. guttatus* complex are being prepared for study to compare the findings in two groups of species belonging to the same genus.

## PHOTOSYNTHESIS AND RESPIRATION IN TWO CLONES OF *Mimulus*

John P. Decker<sup>1</sup>

A study was made of the effects of temperature and CO<sub>2</sub> concentration on rate of CO<sub>2</sub> uptake and evolution by intact leaves of two clones of *Mimulus*. Clone 6546-5 is a plant of *M. cardinalis* from Los Trancos Creek near sea level on the Pacific coast, San Mateo County, California. Clone 6546-3 is an F<sub>1</sub> hybrid between 6546-5 and a plant of *M. lewisii* from near the Timberline transplant station in the Sierra Nevada at 10,500 feet altitude.

The apparatus consisted of a leaf chamber, an air-recirculating pump, and a recording infrared gas analyzer arranged as a closed system. The analyzer recorded continuously the concentration of CO<sub>2</sub> in the system. A change of concentration was considered a direct measure of uptake or evolution of CO<sub>2</sub> by the plant material enclosed in the chamber.

The routine procedure was as follows: The intact tip of a branch, with one pair of opposite leaves, was sealed in the chamber and left at standard illumination (2000 foot-candles) and a constant predetermined temperature (20°, 30°, or 40° C) for 15 to 30 minutes before measurements were begun. Then CO<sub>2</sub> was added to raise the concentration in this closed system to about 450 to 500 parts per million (ppm). The subsequent smooth and uniformly decelerated decrease of CO<sub>2</sub> in the system was recorded. When it reached a conveniently low point, CO<sub>2</sub> was again added and a duplicate measurement was made. This time concentration was allowed to fall to the compensation point, that is, to that low concentration of CO<sub>2</sub> of the air surrounding the leaf at which there was no net gain or loss of CO<sub>2</sub> by the leaf. Then a scrubber bottle of aqueous NaOH was put in the system, and CO<sub>2</sub> was reduced to less than

<sup>1</sup> Visiting investigator, on leave from Rocky Mountain Forest and Range Experiment Station, Forest Service, U. S. Department of Agriculture.



50 per cent of the compensation concentration. The scrubber was removed, and the subsequent increase of concentration (apparent respiration) was recorded. This procedure was duplicated. The chamber was darkened, and duplicate determinations of  $\text{CO}_2$  evolution were made over the low to 300-ppm range. The whole procedure was repeated at the three temperatures before the leaves were removed for the measurement of their area. Six pairs of leaves on three propagules of the hybrid 6546-3 (all of the same clone), and six pairs on five propagules of 6546-5, were used.

Rates of  $\text{CO}_2$  uptake and evolution were computed from the slope of lines drawn tangent to the recorded curves at selected  $\text{CO}_2$  concentrations. Leaf area was computed from the weight of a piece of aluminum foil cut to the pattern of the leaf. Results are summarized in figures 15 and 16. The confidence ranges shown were computed from the error terms of analyses of variance; they can be interpreted as follows: if the experiments were repeated with the same or similar material, mean values would be expected to fall outside the ranges shown not oftener than once in 20 trials.

Within each set, the curves for apparent photosynthesis (consumption of external  $\text{CO}_2$ ) are nearly parallel. This fact suggests that total photosynthesis (sum of both external and endogenous  $\text{CO}_2$  consumed) may have been unaltered by temperature change within the ranges used, that it can be represented by a single straight line parallel to the others and originating somewhere above 0, 0, and that the differences shown between the curves for apparent photosynthesis probably result from the effect of temperature on respiration during photosynthesis.

With the parent plant,  $\text{CO}_2$  evolution in darkness at 300 ppm was consistently less than evolution in light below the  $\text{CO}_2$  compensation concentration. A few measurements were made of  $\text{CO}_2$  evolution in darkness at the same low concentration

range. The rates were nearly equal to the comparable evolution rates in light. A more critical study of  $\text{CO}_2$  evolution in this concentration range will have to await refinement of the apparatus.

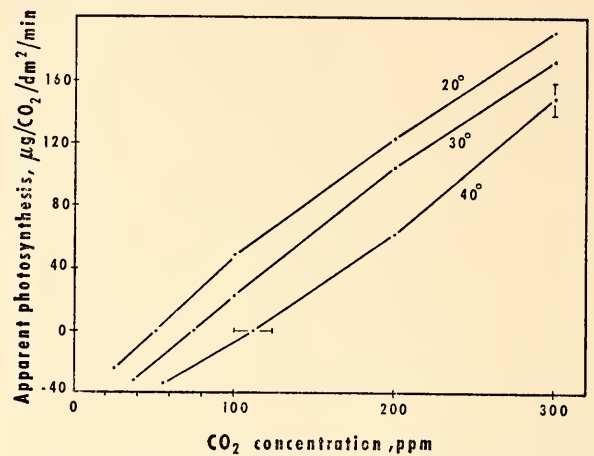


Fig. 15. The effect of  $\text{CO}_2$  concentration and temperature on apparent photosynthesis ( $\text{CO}_2$  uptake) of a clone of *Mimulus cardinalis* (plant 6546-5). Points at  $y=0$  are means of 6 observations; all others are means of 12. Confidence ranges are 5% tse. Values of  $y$  less than zero are of  $\text{CO}_2$  evolution in the light (apparent respiration).

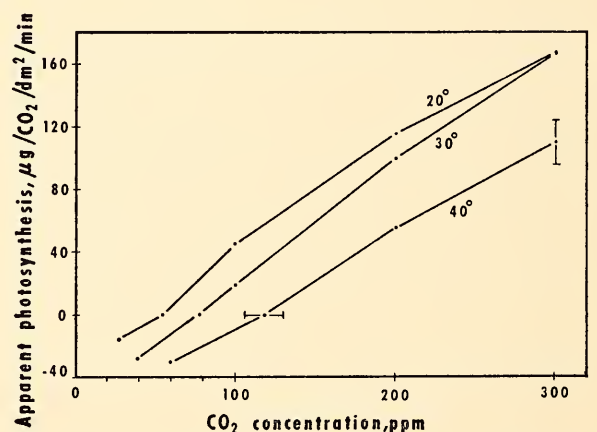


Fig. 16. Same as figure 15, but for the  $F_1$  hybrid *M. cardinalis*  $\times$  *lewisii* (plant 6546-3).

#### THE EFFECT OF TEMPERATURE ON THE REACTION OF PLANTS TO ULTRA-VIOLET RADIATION

Ursula Brodfuehrer

In order to analyze the influence of environment on the reaction of plants to ultraviolet (UV) radiation, open-air investigations at Timberline station were combined with investigations under controlled environments at the Earhart Labo-

ratory of the California Institute of Technology. A need for such investigations was established in 1950 in the Alps by observations made during different times of the growing season. Although UV is usually considered a growth inhibitor, these observations indicated that it may also act as a stimulant. Analysis of the climatic conditions showed that differences in the growing temperature might have caused the different reactions to UV. The sensitivity to UV also differed among varieties, clones, and even different parts of a single plant.

Investigations at Timberline were conducted from the middle of July to the middle of September 1956. Temperature conditions were surprisingly uniform throughout the growing period. The nightly minimum was always near freezing, and the day temperatures, measured 60 cm above the ground, stayed at 18° to 20° C from 1 hour after sunrise to 1 hour before sunset. The plants grew in hotbed-like flats. Five of the flats had open side walls and belonged to a cold series with temperatures similar to open-air conditions; another group was enclosed and had temperatures about 6° C higher. The temperature 1 cm deep in the soil, and in the air layer immediately above it, was on an average 26.5° in the cold series and 33.1° in the warm series between the hours of 9:30 and 17:30. The nightly average between 17:30 and 9:30 was 4.1° in the cold and 9.0° in the warm series. The side walls of the enclosed beds were made of UV-absorbing Plexiglass. The top windows were of different combinations of UV-absorbing Plexiglass, UV-transmitting polyethylene, and wire mesh. By these means, members of each series of five beds were given relative UV dosages of 100, 66, 33, 16, and 0 per cent in the region between 280–290 and 340–360 m $\mu$ . The intensity of the total radiation in each bed was 80 per cent, and the maximum UV intensity was about 70 per cent of that in the open.

Investigations in the Earhart Laboratory

ran from October to December 1956. Growing conditions, with day temperatures as the independent variable, reproduced the climate of Timberline as nearly as possible. From 8:00 to 16:00, groups of plants were kept in three different greenhouses with air temperatures of 17°, 23°, and 30° C. Because of heating by sun radiation, the temperature of the soil and of the air layer immediately above it generally increased 10° in the 17° greenhouse, 7° in the 23° greenhouse, and 4° in the 30° C greenhouse. The actual growing temperature of young and of rosette-like plants, therefore, was higher than the temperature of the air. From 16:00 to 8:00, all plants were kept in a constant-temperature room at 7° C. This room was lighted by fluorescent lamps from 16:00 to 24:00, and was totally dark from 24:00 to 8:00.

The total radiation in the greenhouses and in the room with artificial illumination had a short-wave limit between 300 and 320 m $\mu$ , thus containing practically no middle-wave UV. The supplementary UV sources were two 40-watt Westinghouse fluorescent sunlamp tubes whose continuous spectrum is very similar to that of the sun in the UV region. Their short-wave limit is 275 m $\mu$ , with a maximum at 310 m $\mu$ . In each of the three temperature groups, the plants were further subdivided into four groups which received various amounts of UV. The control group was not exposed; the other three groups received 3, 6, and 12 minutes' exposure a day, with the lamp at a distance of 35 cm. Considering the slight differences between the spectra of sun and lamps and the lack of really satisfactory measuring instruments, only relative information about the UV amounts is given. In the open-air investigations, the UV amount was considered higher in the high Sierras (3000 meters) with 87 per cent relative duration of sunshine than that in the Alps (1500 meters) with 60 per cent relative duration of sunshine. In the Earhart Laboratory, UV dosage in the same range as in the



high Sierras was attempted with the sun-lamp tubes.

The plants used were *Arabidopsis thaliana* var. *Catania*, whose reaction was known from the Alps in 1950. In addition, alpine plants were collected at Timberline station: two clones of *Mimulus tilingi*, small seedlings of *Muehlenbergia filiformis* and *Achillea lanulosa* ssp. *alpicola*, and one clone of *Antennaria alpina* var. *media*. The hybrid *Mimulus guttatus* × *tilingi* came from the collection at Stanford.

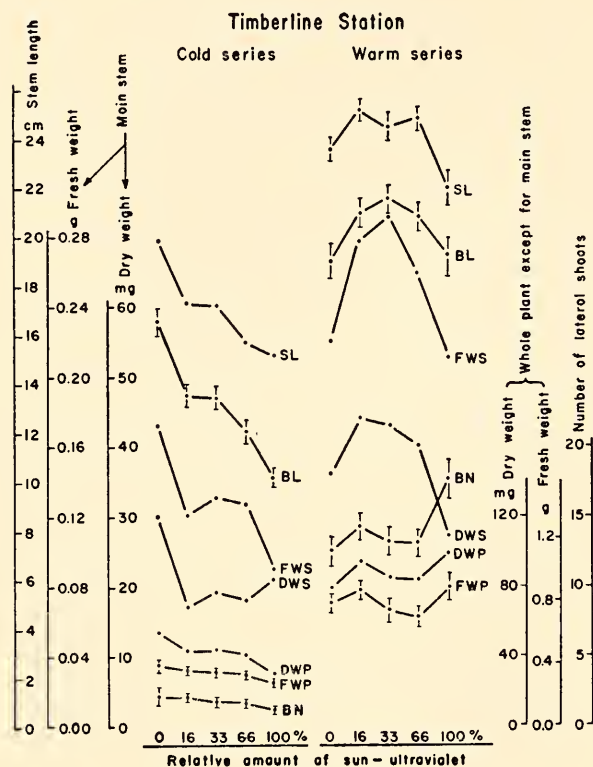


Fig. 17. Temperature-UV effect on growth of *Arabidopsis* at Timberline.

An example of the behavior of the plants at Timberline is given in figure 17. Curves for the cold series of *Arabidopsis* are plotted on the left, and those for the warm series on the right. Although the plants in the cold series show increasing inhibitions with increasing UV dosage, the best development in the warm series occurs at a medium, or even the highest, UV intensity. In this warm series the curves for fresh and dry weight (FWS, DWS) and length of main stem (SL) and side branches (BL) peak between 16 and 33 per cent UV. The curves for the number

of side branches (BN) and dry and fresh weight of the rosettes (FWP, DWP) show greatest stimulation at 100 per cent UV. Slight deviations from the general trend of the curves can be attributed to small temperature variations among the beds of each series. *Antennaria alpina* var. *media* reacted similarly to *Arabidopsis*, but not as distinctly, showing small inhibitions in the cold series and small stimulations in the warm series. Like *Arabidopsis*, the annual grass *Muehlenbergia filiformis*, grown only

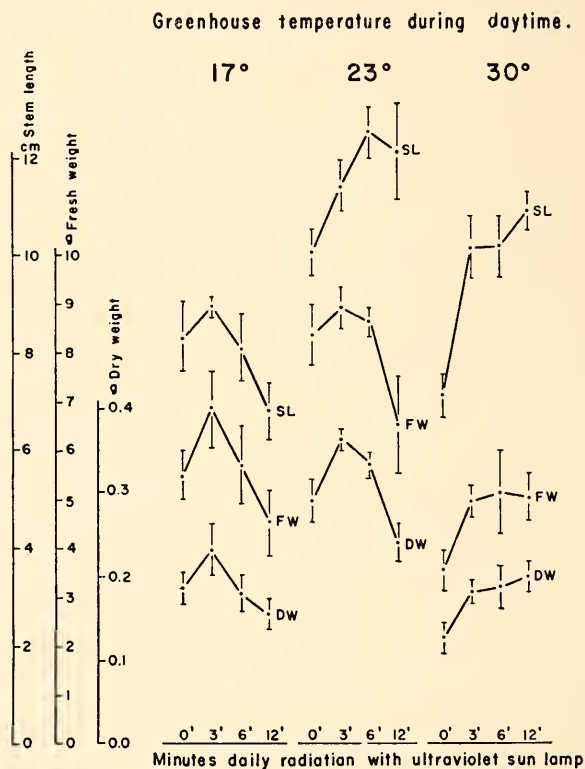


Fig. 18. Temperature-UV effect on growth of *Mimulus tilingi* at the Earhart Laboratory.

in the warm series, clearly showed stimulations, and *Achillea lanulosa* ssp. *alpicola*, grown only in the cold series, showed inhibitions. Clone 1 of *Mimulus tilingi* and the hybrid clone of *Mimulus tilingi* × *guttatus* did not show significant differences. Clone 2 of *Mimulus tilingi* entered the investigations too late for any results.

In the Earhart Laboratory, clone 2 of *Mimulus tilingi* gave the most pronounced responses (fig. 18). In the 17° greenhouse, maximal growth occurred with 3 minutes of UV radiation per day, in the 23° greenhouse with 3 or 6 minutes, and in the 30°

greenhouse with 6 or 12 minutes. The differences between the extreme values of the stem length are highly significant ( $t=3$ ) in all greenhouses. The difference between extreme values of fresh and dry weight are highly significant in the 30° greenhouse and significant ( $t=2$ ) in the other two greenhouses. Clone 1 of *Mimulus tilingi*, *Mimulus tilingi* × *guttatus*, and three successive seedlings of *Arabidopsis* showed behavior similar to that of clone 2 of *Mimulus tilingi*.

The investigations at Timberline and in the Earhart Laboratory explain the seasonal variation of UV response found in the Alps. They show that at higher temperatures UV has a stimulating effect on plant growth that can be as great as 40 per cent. They show, further, that this stimulating effect at higher temperatures is strongly dependent on the amount of UV, and is exhibited only when dosages are as small as those of natural open-air conditions.

#### STUDIES ON DIFFERENTIAL SELECTION IN *Mimulus*

Malcolm A. Nobs and William M. Hiesey

Little information is available on the selective effects of contrasting climates during early seedling stages on a series of altitudinal races. The *Mimulus cardinalis* complex is particularly well suited to this kind of study because of the very large numbers of seeds produced each year by a single individual, and because the seeds, being very small (ca. 0.5 mm × 0.2 mm), carry little reserve food material. Immediately after germination the tiny seedlings are highly dependent upon a reasonably favorable environment for their survival.

In test plantings at Timberline, a population of 700 seedlings of coastal *M. cardinalis* that had germinated at the station during the summer was completely eliminated after the following winter. By contrast, 40 per cent of a corresponding seedling population of a race of *M. lewisii* native in the vicinity of the alpine station and exposed to the same conditions sur-

vived. Moreover, at Stanford little seedling loss is experienced with coastal *M. cardinalis* sown and grown there during the course of a year, whereas seedlings of alpine *M. lewisii* under similar circumstances show only about 10 per cent survival.

Among segregating F<sub>2</sub> seedlings of reciprocal crosses between coastal *M. cardinalis* and alpine *M. lewisii* grown at Stanford, distinct differences were observed in the phenotypes of surviving individuals between sowings made during summer as compared with sowings from the same seed lot made during winter. In the summer-sown lot of F<sub>2</sub>, where germination took place in a greenhouse in which maximum daily temperatures often reached 100° F or higher, the frequency of plants having orange to vermilion flowers resembling the coastal parent was 16 to 22 per cent higher than in winter plantings where maximum daily temperatures seldom exceeded 80° F. In the winter plantings a proportional increase in frequency of pink-flowered types resembling the alpine parent was observed. Among mature F<sub>2</sub> plants grown at Stanford over a 3-year period, the elimination of orange to vermilion-flowered types was 25 per cent less than among the pink-flowered fraction.

These studies suggest that there is a strong differential elimination during seedling stages among genetically distinct forms exposed to contrasting climates, and that there is genetic linkage between conspicuous morphological characters, such as flower color, and physiological qualities that are of importance for survival in different climates.

Additional genetic information from F<sub>3</sub> populations grown at Stanford during 1957 that were derived from F<sub>2</sub> individuals representing a wide range of phenotypes from coastal-like to alpine-like recombinations provides some support for these conclusions. In F<sub>2</sub> and F<sub>3</sub> generations there is significant genetic coherence between factors governing flower color, corolla shape, and the relative length of style and anther filaments. These flower characters



strongly determine which agents can pollinate *M. cardinalis* as compared with *M. lewisii*, as described in Year Book 53, pages 157–159. Linkages between leaf and flower characters have thus far not been detected.

The genetic data also indicate that most characters distinguishing the coastal *M. cardinalis* from alpine *M. lewisii* are governed by systems of multiple genes similar to those found in altitudinal races of *Potentilla glandulosa* (cf. Year Book 47, pp. 106–110). An exception to the multiple genic inheritance is the relatively simple inheritance of the conspicuous yellow chromoplasts in the upper epidermis of the petals of *M. cardinalis* absent in *M. lewisii*, first discovered by Vickery (cf. Year Book 50, p. 119). *Mimulus lewisii* carries a dominant gene that suppresses the development of the chromoplasts in the  $F_1$  and  $F_2$  progeny. This character therefore segregates in a simple ratio of 3 pink:1 yellow. Superimposed on this segregation of a major gene governing petal color, however, are segregations for other pigments and pigment patterns that are governed by complex multiple genic systems that appear to be partially linked.

Further work is now under way designed to identify linkages between morphological and physiological characters that affect survival in various kinds of climate. Another purpose of the work is to develop hybrid recombinations that can be tested at the altitudinal transplant stations and can provide significant materials for use in detailed physiological studies. Samples of  $F_2$  seedlings germinated and subjected to climatic selection at the three altitudinal stations will be cloned and transplanted to all three stations to determine their survival capacity in these contrasting environments. Individuals of particular interest can then be subjected to further genetic analysis and to physiological studies, together with the original parental forms from contrasting altitudes.

#### STUDIES IN *Achillea*

Malcolm A. Nobs and William M. Hiesey

An understanding of the cytogenetic relations between the diploid species of *Achillea* appears to be necessary for a theory of the evolution of the widespread and diverse polyploid forms of the genus found both in North America and in Europe. As mentioned in Year Book 54, pages 182–183,  $F_1$  hybrids between diploid species were found to be sterile, but during the current year unexpected success has been experienced in obtaining some  $F_2$  progeny from the cross *A. asplenifolia* Vent., having pink flowers, by *A. tomentosa* L., having yellow flowers. Both these very distinct species occur in central Europe, and have  $n=9$  chromosomes. The  $F_1$  hybrid has white flowers.

An intensive search for the few viable seeds among a large number of  $F_1$  plants from this cross, most individuals of which were treated with varying dosages of colchicine, has resulted in a population of approximately 300  $F_2$  plants now flowering in the Stanford garden. These plants show many kinds of recombinations of characters of the parental species: flowers varying from pink, as in *asplenifolia*, to pure white, as in the  $F_1$ , and to deep yellow, as in *tomentosa*; leaves ranging from green to tomentose, from wide to narrow, and from deeply serrate to highly pinnate; growth habit from low decumbent dwarfs to taller ascending forms; and vigor ranging from very weak subnormal types to plants fully as vigorous as the parent species.

Cytological study of the chromosome numbers and of meiotic pairing in these plants is now under way. If any of the  $F_2$  progeny should prove to be tetraploids, they will be used for crossing with tetraploid species both from Europe and from North America. It may also be possible to synthesize an array of new polyploid derivatives starting simply with the two contrasting diploid species.

Studies of  $F_1$  and  $F_2$  populations from crosses between contrasting altitudinal and latitudinal races, both tetraploid and hexaploid, as outlined in Year Book 51, pages 122-124, are being continued. Of special interest are the response patterns of cloned transplants from an  $F_2$  population between a tall form of *A. borealis* from the San Joaquin Valley in California and a dwarf form of the same species from Kiska Island in the Aleutian chain. Although this  $F_2$  population exhibits extreme segregation among its individuals, it is, on the whole, outstandingly vigorous at Mather as compared with both the parental forms, but at Timberline a small although significant fraction is able to survive despite heavy elimination of the great majority of  $F_2$  plants. In this alpine environment the parental San Joaquin Valley race is completely eliminated, whereas the parental Kiska race shows better than 50 per cent survival.

In the current studies on *Achillea*, contact is being maintained with Dr. Friedrich Ehrendorfer at Vienna, Austria, who, as a visiting fellow to the Department during 1952, started an active program of study both on the European and on the North American forms of *Achillea* (cf. Year Book 51, pp. 125-130).

#### CONTRASTING TOLERANCE RANGES OF APOMICTIC SPECIES AND HYBRIDS OF *Poa*

Jens Clausen, William M. Hiesey, and  
Malcolm A. Nobs

The hybrids and parental species of the bluegrass genus *Poa* offer unique opportunity to conduct transplant studies on a continental scale, because the seeds of each apomictic hybrid and parent line of this genus belong to a hereditarily constant although highly heterozygous clone. Transplantation by seed clones is a much simpler process than transplanting ramets of live plants.

Through the U. S. Agricultural Research Service, the U. S. Soil Conservation Serv-

ice, and the altitudinal stations of the Department in California, it has been possible to establish 46 seed clones of parents and hybrids at 14 stations throughout the United States. The stations were listed in Year Book 54, pages 172-174. Exceptionally dry years prevented planting at Stillwater, Oklahoma; Manhattan, Kansas; Madison, Wisconsin; and Ephraim, Utah; but the strains were established successfully at the other stations.

During June 1957, five stations in the northwestern part of the United States were visited by Hiesey and Nobs: Corvallis and Pendleton, Oregon; Lind and Pullman, Washington; and Aberdeen, Idaho. In May, Clausen visited the plantings at Franklinton, Louisiana; Columbia, Missouri; Lexington, Kentucky; Blacksburg, Virginia; State University, Pennsylvania; Purdue University, Indiana; and the University of Minnesota, St. Paul.

A fair number of the strains have also been grown at seven stations in Sweden ranging from 55° to 68° N latitude. The results of this experiment at extreme northern latitudes have been reported in a paper by Drs. Axel Nygren and Erik Åkerberg (*Ann. Acad. Regiae Scient. Upsaliensis*, 1, 53-69 [1957]). A group of strains is also being tested by Dr. Paul Solberg at the Norwegian Experiment Station of Agriculture in the Mountain Districts of Volbu, in central Norway. The Poas are being grown at Volbu at 500 meters altitude, and at Berset above tree line at 1000 meters. A third experiment in Europe is being conducted at the Scottish Plant Breeding Station, Pentlandsfield, near Edinburgh, Scotland. Drs. J. W. Gregor and Patricia Watson have arranged a comparative experiment of thirteen hybrid lines derived from the cross *Poa ampla*, Kahlotus, × *P. pratensis*, Athabaska. As was mentioned in Year Book 55, page 238, five of these hybrid lines were selected at Pullman, Washington, two in Scotland, and six at Stanford. In Scotland the parental strains and their hybrid lines are space-planted



and replicated ten times. The data from Scotland will be compared with those of the same strains grown at Pullman, Washington, and at the three altitudinal transplant stations, Stanford, Mather, and Timberline. Although it may require another year or two for the response patterns of these strains at the various stations to be fully determined, it is already possible to draw certain major genetic-ecologic conclusions from this unique experiment.

Several derivatives of each hybrid combination are included in this experiment. They were selected mainly in the second hybrid generation, so that the opportunity existed for genetic recombination to take place between the parental genomes. It was nevertheless found that hybrid derivatives of the same interspecific cross followed very much the same response pattern in the major climatic regions, although minor differences between strains from the same cross were evident. The genomes of the contributing parents therefore appear to have major influence in determining the over-all responses of the hybrid strains. This fact suggests that the individual genes of the parental species are not being thoroughly recombined in the second hybrid generation.

For example, all ten hybrid derivatives of *Poa ampla*, Albion,  $\times$  *P. pratensis*, Mather, are weak or die in the states east of the Missouri River but have excellent vigor in the Pacific Northwest. The central and eastern states are characterized by warm summer nights, whereas in the western states the nights are generally cool. Hiesey found (*Am. J. Botany*, 40, 205–221 [1953]) that in the controlled greenhouse experiments the Albion strain of *ampla* responded best under cold-night conditions, whereas the Mather *pratensis* was tolerant to a great range of conditions. In the regional transplant experiments this *Poa ampla* parent is highly successful in its native Pacific Northwest, but unsuccessful in the eastern states. In contrast, Mather *pratensis* is fairly successful both east and

west. The hybrid group derived from this combination therefore follows the *ampla* parent in its range of tolerance, although minor differences exist between the responses of the individual strains in the group.

The same individual of *Poa ampla* crossed with the far northern form of *Poa pratensis*, subspecies *alpigena*, has yielded very different kinds of hybrid progeny. They are all moderately tolerant of the climates of the states east of the Missouri River. In the controlled greenhouse experiments cited above, it was found that the Lapland parent, native to a climate where the sun does not set during a part of the growth period, tolerates warm-night conditions, even when combined with fairly warm days (Year Book 50, plate I, facing p. 102). The Lapland strain from 68° N latitude suffers at southern latitudes, although it can be grown out-of-doors at Stanford at 38° N, and survives fairly well in the warm-night area of Purdue University at 40° N, and at St. Paul at 45° N. Morphologically, the *alpigena* form has a dominant influence that is evident in all its hybrids. It appears also to have a fairly dominant physiological influence, for the *ampla-alpigena* strains survive with fair vigor where combinations between the same plant of *ampla* and Mather *pratensis* succumbed.

The *ampla-alpigena* strains have an unusual latitudinal range of tolerance. They survive moderately well even as far south as in Louisiana at 30° N, although they are unable to flower there, and on the west coast of North America they attain full development from 34° to 49° N. In Norway they are highly successful at 61° N, and under the short summer nights at 65° N at Öjebyn, Sweden, they develop into plants about a meter in height. The substitution of the *alpigena* genome for that of Mather *pratensis* has, therefore, a spectacular effect.

A third group of *Poa ampla-pratensis* strains are progenies of the cross *Poa*

*ampla*, Kahlotus,  $\times$  *P. pratensis*, Athabaska, mentioned above. This cross has given rise to 19 new lines, 6 selected at Stanford, 5 at Pullman, 2 at Edinburgh, and 6 at Volbu, Norway. The 5 selected at Pullman were included in the tests throughout the United States; they constitute a group that succeeds fairly well both in the warm-night region east of the Missouri and in the far western regions with cool nights. The *ampla* parent of this cross came from a somewhat lower altitude than the Albion strain, and in the coastal regions of the Pacific states the Kahlotus strain tends to surpass Albion, whereas in the central and eastern states Kahlotus is even weaker than the Albion. The Athabaska strain of *Poa pratensis* comes from the high-latitude parklands of central Canada; it is an early-blooming selection that tolerates warm nights and succeeds over a great range of environments, west and east, although in many places it is very stemmy. The five hybrid strains are highly diverse, but they are among the most successful hybrid lines both in the western and in the central and eastern states, where several of the Kahlotus-Athabaska derivatives surpass the Athabaska *pratensis* parent.

A fourth group includes hybrids derived from crosses between the California bluegrass, *Poa scabrella*, and three highly different strains of *Poa pratensis*, namely, the Athabaska, Mather, and Leevining, the last from the California desert plateau region. *Poa scabrella* is a winter-active, summer-dormant, highly stemmy bunchgrass, but six apomictic hybrid lines selected in the F<sub>2</sub> generation represent very diverse morphological types ranging from a low sod type to a tall, bunchy range type. Com-

mon to all is an ability to start growth under fairly low temperatures and to flower early, influences from the *scabrella* genome. The two *scabrella* parental lines die in all the northwestern, central, and eastern environments, being successful only in the California Mediterranean-type climate. Although the *scabrella* genome is by itself completely unsuccessful over the greater part of the areas tested, it has contributed physiological influences in all these otherwise morphologically diverse hybrid lines as expressed in an ability to flower early and in an increase in vigor in certain environments over that of the *pratensis* parent. Moreover, the *pratensis* parents suffer more from dry periods than the *scabrella-pratensis* combinations, so that the *scabrella* genome appears to add a measure of drought resistance to its hybrids.

In conclusion, it has been found that each hybrid combination constitutes a family of lines that is physiologically distinct, and has its own characteristic range of tolerance. The over-all ecological response of such a family of lines depends upon the combination of genomes that have contributed to it. A certain genome may be ecologically the most influential in one cross but subdued in another. Although in certain regions a species may be unable to survive, its genome combined with that of another species may nevertheless add some physiologically important traits that increase the vigor and success of the hybrid over that of the nonhybrid. In such a combination, the genome of a species may therefore be effectively used over a territory that far exceeds the natural range of the species that contributed it.

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# DEPARTMENT OF EMBRYOLOGY

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*Baltimore, Maryland*

JAMES D. EBERT, *Director*



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## INTRODUCTION

The late George L. Streeter, second Director of the Department of Embryology, reviewed in his annual reports only investigations that were completed and published during the report year. Thus, in his own words, they dealt entirely "with past and already cold events." He did so under the conviction that, although ambitions for the future and work in progress are forceful stimuli to endeavor, they are properly the concern only of the investigators themselves. Undeniably, the affairs of research can be predicted only to a limited degree, for opportunism often plays a large role in discovery, and the investigator must be alert to advantageous alterations in course. It happens, however, that the Department has entered a period characterized by exploratory forays and scouting ventures to determine the feasibility of new research programs. The Director, whose function it is to record the progress of research in the Department, believes that the reader should be permitted to share in the growing enthusiasm of the group and occasionally in the difficulties that are being met. The following report, therefore, will not deal entirely with completed investigations and published data. Such information will provide the matrix for the discussion, but work in progress will be considered if it is believed to be of general scientific interest. Emphasis will be placed upon the impact of new ideas and techniques and the development of neighboring sciences on the rapidly evolving field of embryology. Thus the annual report will afford an opportunity to examine critically several aspects of embryology, and occasionally to hark back to the early definitions of a concept and trace its expansion, leading up to an analysis of its present-day scope and its relation to other disciplines.

The advent of a new Director has not resulted in abrupt or dramatic changes in the research program of the Department of Embryology. In our work, we are

guided by a policy that has evolved through four decades. According to the scientific charter of the Department formulated by Franklin P. Mall and adhered to rigorously by George L. Streeter, the principal objective was the study of the human embryo; nevertheless, individual staff members like Warren H. and Margaret R. Lewis and Carl G. Hartman spearheaded research in the areas of experimental cytology and reproductive physiology, respectively. The third Director, George W. Corner, recognizing the responsibility of the Department not merely to keep abreast of the field but also to lead the way in attacking significant problems, placed increasing emphasis on chemical and experimental embryology. It is in this direction that the Department continues to evolve.

Our objective has been stated as the study of the mechanisms underlying cellular differentiation, growth, and morphogenesis, using whatever species of animal or microorganism provides the most favorable experimental material. It seems good generalship at this time to exclude from our purview the analysis of the development of higher plants. This restriction would not be taken seriously, however, should a compelling idea arise that might be tested more directly or simply in a plant than in an animal. Moreover, as Waddington has pointed out, previous research has not revealed any general or important dividing line between the early embryo and later stages of development; hence we understand the word *embryology* as referring to all aspects of development.

*It can no longer be stated that our investigations are concerned chiefly with the form and function of the human embryo.* The contributions of the Department to this selected task have been uncommonly fruitful; yet it cannot be denied that this phase of our activities has reached the point of diminishing return. We cannot say of the program (any more than of any other



scientific study) that it is finished, but we must conclude that it has ceased to challenge the interest and professional capacities of the group as a whole. Admittedly, questions remain to be answered; much valuable information is to be gained through the application of chemical and histochemical techniques to human embryos, as exemplified by the recent studies of Claude Villee and Arthur T. Hertig and his co-workers. But the challenging problems in embryology today confront the experimentalist; each thrust forward increases enthusiasm and initiative. This does not mean that the Department's important Collection of Human Embryos must fall into disuse. On the contrary, in fact, the year covered by this report has seen the Collection used more actively than for several years past. The immediate future of human embryology in the Department is clear: we will continue to add to the Collection specimens that show promise of being of value in research; emphasis will be placed on embryos in pre-somite and early somite stages (horizons i to x, ovulation to 24 days), on embryos prepared for studies in neurogenesis, and on embryos fixed for study by the methods of histochemistry and electron microscopy. We do not propose to embark on a program employing these modern descriptive techniques; in response to several requests received during the past year, however, we are preparing to co-operate with other laboratories already actively engaged in these areas of research.

The staff will not include an embryologist whose principal duties center about the Collection; instead we propose to stress the experimental morphology of the principal species used in the chemical and physiological investigations in the Department. We expect that when Dr. Mary E. Rawles, an embryologist of great talent, joins the Department late in 1957 she will assume leadership in normal descriptive embryology (including human embryology) in addition to continuing her widely recognized experimental studies. It is our in-

tention that the Collection be used actively by qualified visiting investigators, whom we stand ready to assist as far as we are able. This policy should result in a highly varied program, and, owing to the close interplay that exists in a small group, it should also result in a mutually profitable interaction; the learning process should be reciprocal.

The decision to assign human embryology to an ancillary position was not arrived at easily. In the writer's opinion, however, the continued exploitation of such an established area of embryology is not consistent with the objectives of the Carnegie Institution of Washington. Moreover, it does not permit the flexibility of approach that is essential at a time when embryology has assumed a central position among the biological sciences.

The central position of embryology is understood best when it is viewed in relation to biochemistry, cell physiology, and genetics. Evidence from studies of the transformations of genetic type in the pneumococci and in *Hemophilus*, and from bacteriophage infection and transmission of genetic traits, has led geneticists to accept the view that the primary genetic material is deoxyribonucleic acid, or in a few instances ribonucleic acid (e.g. in plant viruses like tobacco mosaic virus). The formulation of the Crick-Watson hypothesis of the structure of deoxyribonucleic acid has led Beadle and others to define the gene as a localized unit of nucleic acid with a specific function, presumed to consist in the determination of the specificity of a nongenic molecule such as a protein. Although no one has taken serious exception to the Crick-Watson model, it must be emphasized that we have only a general plan; a complete analysis of the sequence of bases is essential for a knowledge of the chemical basis of genetic differences. Equally critical is the need for tests of biological specificity of nucleic acids in heredity and functional development, stimulated especially by recent successes in the enzymatic synthesis of deoxyribonucleic acid *in*

*vitro* and the net synthesis of protein by subcellular preparations.

The advance of biochemical genetics has been accelerated by the widespread use of microorganisms, for such material makes possible the study of phenomena that occur with a frequency several orders of magnitude smaller than those investigated in higher forms. A number of genetic studies with microorganisms have been motivated by their application as models of cellular differentiation. Such studies are helpful in suggesting working hypotheses that should be considered for development, but, as Lederberg has stressed, which concepts are correct must be learned by asking questions of embryos rather than of microorganisms. Clearly, it may be feasible to analyze the genetic differences among differentiated tissues with no less exactitude than is possible for the clones of mutant microorganisms. Here again we are confronted with a lack of knowledge of exactly how the genetic material interacts with other parts of the cell. This fundamental problem of biology is being attacked from many sides, but it is essentially an embryological one. A part of the research of the Department is concerned with the study of somatic cell variation in development. Much of the dogma of experimental embryology stems from studies of the behavior of embryonic tissues under experimental conditions. Whereas the experiments have been carried out on tissues, the conclusions often relate to the cells of which the tissues are constructed. A transplanted tissue containing many cells may be able to continue in its normal developmental pathway only because of interactions among its constituent cells. In brief, then, a major objective of embryology should be to elucidate the roles played in cytodifferentiation and histogenesis by interactions among the cells comprising a tissue and to determine the nature of such interactions as may be found.

As will be made clear, another major objective of our program of investigation is the elucidation of the mechanisms regu-

lating differentiation at the chemical level. Recent progress in biochemistry is yet to make its full impact on studies of development. As an example may be cited the failure to demonstrate unequivocally whether enzyme induction and repression play a significant role in regulating differentiation and growth, despite the fact that these well demonstrated phenomena in microorganisms do present attractive models of differentiation. There are good grounds for the view that induction is not essential for the production of all enzymes; therefore, the balanced system of enzyme induction and repression is pictured as a control mechanism, rather as a *sine qua non* of enzyme biogenesis. The changing nutritional requirements of embryonic cells and tissues and the relation of these demands to the development of tissue-specific molecules clearly offer attractive areas for investigation. As in all investigations, the use of chemical and immunochemical techniques in studies of development requires that meaningful questions be asked; it no longer suffices to draw up a chemical inventory of the embryo.

With the arrival of the new staff members whose appointments were announced in Year Book 55, and the completion of a modest program of renovation of the physical plant, the Department has begun to function effectively as a department of experimental embryology. In the belief that neither the methods of classical experimental embryology nor those of conventional biochemistry are likely to suffice to bring a solution to the mechanisms of development, each staff member has undertaken to carry on an evaluation of the most creative activities he can pursue within the continuously expanding range of his competence and interest. The reader will note the emphasis on the personal nature of the research; creative research is an individual undertaking. We have no directed, highly organized research teams, yet several collaborative efforts have sprung up spontaneously in the stimulus of close associa-



tion with interested research men both within the Department and in other laboratories in which problems of development occupy a prominent position.

During the year there were no serious distractions; no major changes were made in the research staff. On June 30, 1957, Miss Harriet L. Caspari retired from her post as senior technician. She joined the Department in 1927, and during her years of association with the laboratory her devotion, painstaking care, and thoughtfulness have contributed to many important investigations.

Five Visiting Fellows carried on research in the Department. Dr. Vincent J. De Feo completed his second year as a Fellow of the U. S. Public Health Service and prepared to take up a new position as Assistant Professor of Anatomy in the University of Illinois College of Medicine. Dr. Seymour Katsh continued research in consultation with Dr. David W. Bishop during the second year of a three-year program conducted under the sponsorship of The Population Council. Dr. Malcolm S. Steinberg, Fellow of the Carnegie Institution of Washington, will continue his investigations in the Department for a second year. During the late spring and summer of 1957, Dr. Jacques Mulnard, of the Université de Bruxelles, carried on research in collaboration with Drs. Royal F. Ruth and James D. Ebert. The outcome of his intensive and fruitful studies will be described, as will the investigations of the other Fellows, under the appropriate headings below. Dr. Mulnard visited the Department as a Fellow of the Rockefeller Foundation. In June 1957, Dr. Louis E. DeLanney, Professor of Biology at Wabash

College, began a productive visit as a Fellow of the Carnegie Institution of Washington, working in collaboration with Dr. Ebert.

All together, exactly fifty visiting investigators from fifteen countries spent from one day to several months in the Department, studying specific topics in the unique material provided by the Carnegie Collection of Human Embryos, making use of the experimental facilities of the laboratory, and observing technical procedures. Obviously, to cite each investigator and his subject is impossible, but mention must be made of those whose studies were sufficiently intensive and productive to warrant description in these pages: Professor E. A. Boyden; Dr. John D. Des Prez; Dr. Chester H. Heuser, Research Associate of the Department of Embryology; Dr. Edward Roosen-Runge; Dr. Bernice Wedum; and Professor Emil Witschi.

The year was one of active and uninterrupted work in the several lines of research established, together with a number of exploratory studies designed to probe pathways for attacking the problems outlined in the foregoing discussion. It is encouraging to observe that these exploratory ventures are not confined to the newly appointed junior members; this report concludes several aspects of research by established members of the staff and presages the advent of new concepts of growing future interest. The flow of published results has been small; the volume of work in progress has been large, and, as the discerning reader will detect, difficult to assess; but it has been an exciting year, and as the new year begins the enthusiasm and initiative have been sustained.

## THE GAMETES

### *Molecular Basis of Sperm Activity*

The present report proves to be a turning point in the program of Dr. David W. Bishop, since it tends to conclude one aspect of the chemistry and physiology of reproduction and to presage the advent of

another. The study of the physiology of the rabbit oviduct, initiated to further our understanding of the tubal environment surrounding the mammalian gametes and embryos during initial stages of development, has been carried as far as seems

feasible for the present. Further mention of this program will be made later in the report.

Developing out of earlier studies of sperm metabolism and a continuing interest in sperm motility, a program was launched to investigate the molecular basis of sperm activity. The initial stages in this study were supported in part by the National Science Foundation. The apparent similarity of the mechanisms of sperm motility and muscular contraction challenges the investigator to isolate and identify the contractile proteins of spermatozoa. It seems likely that by the preparation and study of sperm-cell models and by the extraction and characterization of functional contractile proteins the mechanism of sperm motility can be explored. Furthermore, with the application of immunochemical techniques, a precise comparison of contractile proteins from different sources may be anticipated. Much has been accomplished during the past year, but the ultimate extraction of a functional contractile protein from sperm, as well as a demonstration of its immunochemical similarity to myosin, actomyosin, or other proteins of muscle, or contractile proteins from sources other than muscle, is yet to be realized. Such goals constitute Dr. Bishop's immediate objectives; his long-range objectives are the problems involving the nature of energy distribution, rhythmicity, and control of motility, *in situ*, in sperm.

The study of the mechanism of sperm motility was initiated in the Physiological Institute in the Max Planck Institute for Medical Research, Heidelberg, Germany, under the directorship of Professor Hans Weber. The work was done as an independent investigation but in close collaboration with Dr. H. Hoffmann-Berling, a member of the staff of the Institute.

A technique was devised to prepare cytolized, extracted, mammalian sperm-cell models which, upon the addition of adenosine triphosphate (ATP) or inosine triphosphate (ITP), resume motility not

unlike that of fresh cells. These model systems possess most of the properties of models of muscle fibers, and their behavior indicates the presence in the sperm tail of a contractile protein like actomyosin. Sperm-cell models were investigated in relation to the effective concentrations of ATP and of calcium and magnesium ions; as in muscle, the concentration of calcium appears to be an important factor, especially at supraoptimal levels of ATP. A reliable and objective method of measuring reactivation has been found in the determination of the rate at which the cell models split ATP. Preliminary experiments using high-viscosity media around the reactivated cell models have suggested a method to determine quantitatively a value for sperm activity equivalent to the "maximal tension" of muscle-fiber models.

Repeated attempts to extract *functional* contractile protein from sperm tails have proved unsuccessful thus far. A protein fraction with ATP-splitting activity, a substance similar in several respects to myosin, has been obtained, but Dr. Bishop has been unable to combine this protein with its presumed partner in the contractile process, actin, derived from muscle. A functional contractile protein, however, was extracted successfully from bull testes. Like actomyosin, this protein was characterized by ATP-splitting activity and underwent viscosity change and superprecipitation upon the addition of ATP.

As an adjunct to the study of sperm, the flagella of the bacterium *Proteus vulgaris* were investigated. These isolated organelles show ATP-splitting activity comparable to that of sperm tails, and a study was initiated to extract contractile protein from this source.

#### *Rabbit Egg Coverings: Chemical Nature, Organization, and Role in Implantation*

In Year Book 51, George W. Corner recorded the appointment to the research staff of Dr. Bent G. Böving, and described the first experiments and observations in Dr. Böving's long-range program aimed



at elucidating the mechanisms of attachment, orientation, and implantation of the mammalian embryo. The implanting embryonic tissues offer a striking example of the general phenomenon of tissue invasion. The record of this investigation, which can be followed in successive Year Books and in several publications, is one of continuous progress. The general anatomical, chemical, and physical study of the sequence of events preparatory to and during implantation of the rabbit blastocyst, including the identification and description of the stages involved, has been completed and published. In addition, Dr. Böving has concluded his investigations of blastocyst orientation (polarization) and transport and spacing. The major findings in the study of blastocyst distribution, which have been published in the *Corner Festschrift Volume* of the *American Journal of Anatomy*, were summarized in Year Book 55 and need not be recounted. During the past year, Dr. Böving has continued to work intensively on the mechanism by which the blastocyst adheres to the uterine epithelium. Inasmuch as the study of the interaction between the blastocyst and uterus requires an understanding of the chemical nature and organization of the surface layers of the blastocyst, the investigator was led to an examination of the noncellular membranes of the rabbit blastocyst. With the assistance of Mr. William Duncan, who furnished a series of outstanding histological preparations employing the Feulgen and toluidine blue techniques, Dr. Böving has arrived at the following conclusions, which he presented at the annual meeting of the American Association of Anatomists.

In the ovary, rabbit eggs are surrounded by a hyaline membrane of neutral or weakly acid mucopolysaccharide. The thickness of this layer is approximately 3 microns; its volume is about 0.0001 cu mm. Böving has proposed that this layer, generally referred to as the zona pellucida, be called the *oölemma*. As the egg moves through the oviduct, there is added a hya-

line envelope of acid mucopolysaccharide having a volume of about 0.05 cu mm. For this layer, which has for many years been called erroneously the "albumen" layer, Böving has advanced the name *mucolemma*. By the time eggs have developed into blastocysts and have begun to attach to the uterus, their diameter has increased nearly fiftyfold (to about 5 mm). This expansion would have stretched the zona pellucida too thin for visibility with the light microscope, yet a membrane about 5 microns thick is present. Although this membrane has a volume about a thousand times as great as the true zona pellucida (*oölemma*), and has two layers rather than one, it is often confusingly called the zona pellucida. A clue to the significance of this membrane comes from a study of its two layers: the inner layer corresponds in volume and staining to the tubal contribution, the *mucolemma*. The outer layer, which is slightly greater in volume, differs histochemically, is less homogeneous, and is less regular in thickness, is contributed by the uterus and accumulates on beads retained in the uterus from 2¾ to 7 days *post coitum*. This layer becomes adhesive and, Böving argues, provides the first attachment of the blastocyst to the uterus. Böving proposes that it be called the *gloiolemma* (sticky peel). After the initial attachment of the blastocyst, the outer, noncellular membranes are digested.

The spatial and temporal pattern of their digestion is of interest, since the process appears to be correlated with the hemotropic interaction between the trophoblast and the uterus. Dr. Böving is bringing to completion a detailed treatment of the idea, which he first described in 1952, that the invasion of the uterine epithelium by the trophoblast has, in fact, a hemotropic basis, together with a general evaluation of the requirements for invasion. Such an analysis is possible because early attachments of the trophoblast to the epithelium are both numerous and discrete. Thus one may discern and demonstrate with mathematical rigor what anatomical factors are

consistently associated with invasion and may be presumed necessary, what factors are randomly associated with invasion and presumably have no directive influence, and what factors are avoided and may be presumed unfavorable. Böving found it unlikely that the trophoblast invades in the direction of any stored nutrient or glandular secretion. He held that invasion requires an aggregate of contact by "knobs" of trophoblastic syncytium with the uterine epithelium, preferably on a projecting portion of the epithelium rather than near gland openings. The adhesion tends to occur at epithelial cells that have a blood vessel at their base. Further data are being collected in such a way that morphological and statistical analysis may be derived from the same material, an approach that makes possible an estimate of the advantages and limitations of each method.

During the adhesion process the disintegration of the mucolemma precedes that of the gloiolemma. The changes occur in the vicinity of the syncytial aggregates of trophoblast and are absent in the hemisphere in which these knobs of trophoblast are lacking. Gloiolemma deposited on beads did not appear digested at the stage of pregnancy at which the envelopes of living blastocysts had disappeared. In addition, the membranes of dead blastocysts are not digested concomitantly with those of littermate living blastocysts. These observations suggest but do not prove that the digestion of the noncellular membranes may result from the enzymatic activity of the trophoblastic aggregates.

The question arises whether the concept of hemotropism advanced on the basis of studies with the rabbit can be applied generally, or whether it should be confined to the one species. Although Böving has not explored the comparative aspect of the problem widely, he has mapped the three pertinent specimens in the Department's collection of macaque embryos, and has found the situation similar to that in the rabbit with respect to hemotropism. Statistical study has not been made.

As these well defined studies have proceeded, Böving has continued to give attention to the nature of the chemical transfer between the blastocyst and the maternal circulation, a transfer determined or limited by the membranous epithelial cells of the endometrium, to the regulation of trophoblast hemotropism by ovarian hormones, and to the measurement of the physical forces involved in the adhesion of the trophoblast to the endometrium. Although the findings continue to agree with the working hypothesis that progesterone promotes trophoblast adhesion through augmentation of uterine carbonic anhydrase, the enzyme that catalyzes the interconversion of carbonate ion and carbon dioxide, the evidence is too indirect and scattered to warrant a further elaboration of the theory at this time.

#### *Mammalian Fertilizin*

In 1913 Frank R. Lillie demonstrated the presence of a sperm isoagglutinin (which he termed fertilizin) in the water surrounding sea-urchin eggs. Since that time, many reports have dealt with the occurrence of fertilizins in other invertebrates (and a few vertebrates); moreover, the chemistry and mode of action of the fertilizins of sea urchins have been subjected to intensive study. Little attention has been paid, however, to the possible occurrence of fertilizins in mammalian eggs. In a paper published in the *Journal of Experimental Zoology*, Drs. David W. Bishop and Albert Tyler, of the California Institute of Technology, describe a series of observations and experiments that indicate the presence of a fertilizin-like substance in the eggs of several mammalian species, e.g. rabbits, mice, and cows. When eggs of these species were placed in dilute suspensions of homologous sperm, the sperm were observed to agglutinate, a reaction attributed by the authors to the diffusion from the egg of a substance analogous to the fertilizins of invertebrates. Several lines of indirect evidence suggest the source



of the mammalian fertilizin to be the zona pellucida, the hyaline membrane of neutral or weakly acid mucopolysaccharide surrounding the ovarian egg. The role of the reaction, which was found to be predominantly species-specific, is not clear. The authors point out the similarities between

the invertebrate and mammalian fertilizins but emphasize that to discuss the chemical nature of the mammalian fertilizin and its mode of action would be premature. Although the authors' findings are of interest in themselves, their chief value lies in the further experiments they suggest.

## DIFFERENTIATION AND MORPHOGENESIS IN THE EARLY EMBRYO

### *The Concept of Determination*

During its differentiation, an organ primordium passes through stages of increasing specificity and stability until it finally forms characteristic types of tissues. At some point during this sequence of events, the fate of a given region of the embryo becomes fixed, so that it can be altered only within a restricted range by experimental means. The time at which the restriction is imposed varies from species to species. Thereafter, that region will always develop into one fairly definite end product. The process by which this restriction is achieved is spoken of as the process of determination; classically, the determination of a tissue is defined by its behavior when studied in isolation and in combination with other tissues, using techniques such as explantation and transplantation. From such studies, the conclusion has been drawn, explicitly or implicitly, that at the developmental stages thus pinpointed the cells concerned have reached the degree of synthetic stabilization and autonomy beyond which they can carry out, without the occurrence of developmentally significant interactions with their neighbors, all the further processes relative to their continued differentiation.

Between the experimental evidence and the above interpretation, however, there lies a gap. Whereas the experiments have been performed on *tissues*, the conclusions concern the *cells* of which the tissues are constructed. Is it not possible that a tissue fragment containing a large population of cells may be able to continue in its normal developmental pathways only because of

interactions among the component cells of the fragment? Thus, the tissue fragment might appear to be "determined" while some or all of the cells of which it is constituted are still in a developmentally labile condition.

Dr. Malcolm Steinberg is interested in the mechanisms whereby developmentally significant interactions among the cells of a tissue might contribute to the histogenesis of the fragment considered as a whole. First, there is a lower limit on the size to which a fragment can be reduced and still differentiate normally. Moreover, not only the "size" of a tissue fragment appears important, but also the effective cellular concentration within it. In an isolated fragment, there might be two (or more) distinct cell types, each of which elaborates substances required by the other(s). Taking the simpler example, as long as both are present, histogenesis should proceed normally, but, if the two categories were to be separated, neither could develop. Another, closely related, possibility imposes one additional condition, viz. the requirement for a specific spatial orientation. A tissue might contain several distinct cellular components, each having to be in direct contact with certain of the others in order to develop normally. If the arrangement of the cells in a fragment were to be altered so as to disturb the mutual spatial relations among these components, normal development of the cells, and consequently of the tissue fragment, could not proceed.

For convenience, the theoretical conditions outlined above may be referred to respectively as quantitative, "symbiotic,"

and "ecological" requirements. Quantitative requirements have been established by others for various embryonic systems (one hesitates to single out a few among many, but the work of Drs. Clifford Grobstein and Edgar Zwillig merits special mention) and by Steinberg for the regenerating hydranth of the marine hydroid *Tubularia*. "Symbiotic" and "ecological" requirements of cells have not yet been elucidated fully, but the recent experiments of Drs. Charles Wilde and C. L. Markert dealing with "cross feeding," especially Wilde's demonstration of metabolic interactions in the synthesis of melanin, furnish an example of "symbiotic" action at the tissue level.

If "ecological" requirements exist, one might demonstrate them by reducing the tissue fragment to a cell suspension and forcing the cells to reassociate in altered arrangement. The demonstration of possible symbiotic requirements demands a more exacting approach. It would be necessary not only to dissociate the tissue cells but also to achieve a separation of the discrete categories of cells, one from another, before allowing them to reassociate, either separately or together. Experiments have been started in both these directions. In brief, the central objective of Steinberg's research program is to elucidate the roles played in cytodifferentiation and histogenesis by interactions among the cells comprising a tissue and to determine the nature of such interactions as may be found.

*"Ecological" requirements.* In a series of experiments with gastrulae of the Japanese salamander *Triturus pyrrhogaster*, the prospective blood-island area was isolated and grown in liquid medium. As others have found previously, blood cells, endothelium, mesothelium bounding a coelom, and pronephric tubules are formed in such explants. Similar explants were dissociated through exposure to a chelating agent, ethylenediaminetetraacetic acid, disodium salt (EDTA), and the resulting cells were thoroughly randomized and allowed to

reaggregate before culturing. In these explants, although cell division proceeded normally, histogenesis failed to occur. Beyond the rare appearance of a few melanophores and the development of cilia on the covering epithelium, no identifiable cell types could be found, nor were the internal cells arranged in any recognizable order.

Whereas it is tempting to attribute the failure of differentiation and morphogenesis to the disruption of a previously existing ordered distribution of dissimilar cells within the tissue fragment, other plausible explanations can be adduced. Prominent among them is the possibility that the EDTA has removed from the cells one or more species of polyvalent cation necessary for their continued differentiation but not required for proliferation. Suspicion might center on iron, manganese, or possibly cobalt, all of which are absent from the culture medium. Dr. Steinberg has conjectured also that calcium and magnesium, present in the medium, cannot return to all their normal sites within the cell once they are removed. It may be too ready a supposition, however, to assume that chelating agents exert their effects solely by the removal of polyvalent cations. For example, Kaufmann, McDonald, and their associates in the Department of Genetics have questioned the conventional explanation of the effects of EDTA on chromosomes as denoting degradational action of EDTA through removal of bonding calcium and magnesium. In Year Book 55 they marshal several lines of evidence that indicate an action of the commercial preparation Versene on cellular nucleoproteins, resulting in changes in their gel-like properties, producing mitotic abnormalities in living onion root tips and modification of basophilia. The implication is that Versene has a pronounced effect on cellular ribonucleic acid. The effect is not necessarily a direct one but probably manifests itself through interference with cell metabolism. Experiments are necessary to resolve this question. Nonetheless, recognizing the inconclusive-



ness of the existing data, we believe that progress has been made in the search for "ecological" requirements in the processes of cytodifferentiation and histogenesis.

*"Symbiotic" requirements.* Here we can only outline work just beginning and note two encouraging observations already made. As stated previously, a technique is required for separating discrete categories of cells from one another. The method employed by Steinberg is a modified type of electrophoresis in which particles bearing both positive and negative charges are brought to rest in a stable  $pH$  gradient at a point at which they bear no net charge. Drs. Böving and Steinberg have designed and constructed a line-operated electrophoresis of this type, based on a model described recently by Kolin. If a developing tissue is cellularly heterogeneous, and if the "net isoelectric points" of the differing cells also differ, then the way is open, after dissociation of the tissue into its component cells, to segregate the component cell types by this form of electrophoresis. In the first application of this method to living cells, washed guinea pig sperm were used. The spermatozoa were concentrated electrophoretically into a narrow stratum at a highly acidic  $pH$ . The high temperatures developed in the apparatus, however, killed the sperm. Subsequently, the apparatus was modified to avoid heating of the sample, and a second experiment was performed, this time using a suspension derived from early chick embryos. This suspension contained clusters of cells in addition to the many single cells. Owing to the heterogeneity of the cell clusters, clearly delineated strata might not be expected. Nevertheless, upon passage of the current, an incomplete segregation of the suspension into two bands was observed, a highly encouraging result. It seems wise to restrict application of the method at first to well known material. Therefore, it is proposed to investigate three tissue systems by this method: liver, heart, and blood cells. If the cellular components of any of these tissues can be segregated with-

out suffering undue damage, then attention will be turned to the earlier stages of differentiating tissues in a search for cellular inhomogeneity. Should such inhomogeneity be found, the presence or absence of "symbiotic" relationships among the differing cells could be determined by means of culture techniques, and the possible genetic basis of somatic cell variation might be explored.

### *Chemistry and Physiology of the Developing Heart*

Recent attempts of embryologists to investigate differentiation in specific chemical terms have met with varying degrees of success. Attempts have been made to analyze the changing nutritional requirements of cells and tissues and to relate them to the development of specificity by describing the ontogeny of tissue-specific molecules. A variety of techniques, biochemical, biophysical, and immunochemical, have been combined to yield information of potential value but few conceptual advances.

Our program, which concentrates on the elucidation of fundamental problems about the nature and direction of the chemical reactions involved in embryonic processes, is conceived on broad lines, because we believe that only by approaching the problem from several angles can we accumulate well balanced data on which to base conclusions. There is a definite advantage in having these diversified approaches represented in the Department, since we can derive early benefit from various discoveries and avoid the pitfalls of narrow specialization. A further reward is found in the study of collateral problems and joint programs of several staff members.

*The origin of spontaneous contractility.* Among the many complex problems presented by the developing vertebrate heart, the origin of spontaneous contractility is of special interest to Dr. Robert L. DeHaan. One of the most plausible theories of the mechanism of pacemaker action is that postulated by a British pharmacolo-

gist, J. H. Burn. He and his co-workers have obtained evidence that acetylcholine, synthesized within atrial pacemaker cells, is intimately associated with the "firing" of the atrial contraction. Application of this theory to the embryonic heart should make it possible to determine whether the onset of spontaneity coincides with the onset of production of acetylcholine, or at least with a significant increase in its production.

During the past year, much of Dr. DeHaan's effort has been devoted to refining one of several methods for the assay of acetylcholine sufficiently to measure the minute quantities of that substance to be found in the early tubular chick heart, before and during the onset of contractions. This endeavor has met with only limited success. It would appear that none of the assay techniques, either biochemical or biological, can be made sufficiently sensitive to detect acetylcholine reliably in quantities as small as those calculated to be obtainable from the early heart ( $10^{-10}$  to  $10^{-12}$  gram). A great deal has been learned, however, about the handling and determination of acetylcholine, making it possible to launch a program, now in progress, to determine activity of the enzyme choline acetylase in the early heart. Though the ultimate assay is still that of acetylcholine, much greater quantities can be obtained per milligram of tissue by incubation of the hearts in the presence of appropriate substrates and cholinesterase inhibitors. Thus, it should be possible to determine whether or not choline acetylase activity appears simultaneously with spontaneous contractions, and, if so, whether specific inhibitors of this enzyme prevent the onset of the contractions. Furthermore, experiments should reveal whether the rhythmic beat produced in the heart is due to a "pulsatile" production of acetylcholine or to a continuous production on which is superimposed a cyclic pattern of inhibition or breakdown.

*Morphogenetic movements in cardiogenesis.* A second, collateral study in prog-

ress, which arose out of observations made in the course of the work described above, is concerned with the morphogenetic movements of mesoderm and endoderm associated with heart formation. Dr. DeHaan discovered that, in chick embryos cultured endoderm surface up by the method of New, the paired cardiac primordia could be prevented from fusing by treatment with crystals or solutions of acetylcholine. Under these conditions, two independently beating hearts are formed, one on each side of an otherwise normal chick. Such a "cardia bifida" animal, obtained by treatment with one small crystal of acetylcholine (which dissolves almost immediately when placed on the area pellucida endoderm) is shown in figure 1 as a whole-mount preparation, after 24 hours of culture. (Figure 1 is on plate 1, facing p. 326.)

Present research is designed to explain the mechanism of this effect. It has already been determined that the effect is not specific to acetylcholine. Since this substance is known to be highly labile, "control" animals were treated with the breakdown products of acetylcholine, choline and acetate ion. With both materials cardia bifida was produced. Experiments by L. V. Heilbrunn and others indicate that, at least in some cells, acetylcholine can cause the release of protein-bound calcium from the cell cortex. The calcium is either driven into the cell interior and bound, or is liberated, to be picked up by natural chelating agents outside the cell, such as the amino acids and plasma proteins. Furthermore, acetate ion itself is known to form co-ordination compounds with heavy metals. Therefore, DeHaan suggested that, in the chick treated with one of these agents, normal cell migratory patterns were being modified by sequestration of calcium (and/or magnesium, iron, etc.). Some evidence has been obtained, tentatively confirming this hypothesis. DeHaan has shown that cardia bifida can be produced by treatment with known chelating agents such as citrate, oxalate, and ethylenedi-



aminetetraacetic acid. Work in progress is designed to obtain quantitative information on agents that will produce this effect, and to determine whether the activity of these agents can be offset or modified by previous treatment with heavy metals. Also it should be possible to see whether acetylcholine has a synergistic action with chelating agents, in the production of cardia bifida.

Two other, closely allied approaches to the problem of morphogenetic movements in cardiogenesis are also under investigation. One is the elucidation of the intercellular relations that presumably are disturbed in the production of cardia bifida. Dr. N. T. Spratt, Jr., has recently suggested that intercellular fibers may be responsible for orienting embryonic cells, one to another. Can staining techniques be perfected to demonstrate these fibers and the presumed effects on them by the agents causing double heart formation? Can other modifications of cell-to-cell bonds be observed? Answers to these questions are being sought.

The second approach in the study of the morphogenetic movements in cardiogenesis is concerned with observing the movements of the mesodermal cardiac primordia, relative to the underlying endoderm. To this end, a series of carbon marking experiments has been undertaken by a senior biology student from The Johns Hopkins University, Mr. Charles J. A. Schulte, III, under the guidance of Dr. DeHaan. Mr. Schulte will continue his work during the coming academic year supported by an undergraduate fellowship of the National Science Foundation. To avoid the difficulties of interpretation of movements of carbon particles, often inherent in this type of work, crucial movements will be followed by means of time-lapse photography, techniques and apparatus for which have been developed over the past year partly in collaboration with Dr. Bent Böving.

*Synthesis of the cardiac contractile proteins.* Any discussion of early cardiac de-

velopment would certainly be incomplete without taking cognizance of the problems of the development of the property of contractility itself. According to Nicholas, Patten, and many others, both skeletal and cardiac muscle become contractile at a stage of histological differentiation much more primitive than that in which the usually accepted microscopic characteristics of muscle can be demonstrated. At the biochemical level, moreover, there is evidence that the specific contractile proteins of muscle are present before striated myofibrils. For example, in a paper published recently in the *Journal of Experimental Zoology*, Dr. DeHaan has demonstrated that protein immunologically indistinguishable from that of fully formed muscle is present in the regenerating urodele limb at least as early as the appearance of the first unsegmented myofibrils, and well before striae occur. And, as Ebert and his associates have shown, cardiac myosin is present in the chick embryo as early as the intermediate streak stage, subsequently being limited to the heart-forming areas at the head-process stage, the time at which cardiac actin can first be detected. A number of questions have occurred to Dr. DeHaan: At what point in their development will the cells of the early heart respond to an outside stimulus by contracting; and what are the biochemical events occurring at this time to allow such a response? More specifically, is this response associated, for example, with the appearance of segmented or nonsegmented myofibrils, and, therefore, with a specific spatial orientation of actin and myosin? Is it perhaps related to the first appearance of an ATPase active meromyosin?

At this point Dr. DeHaan's investigations tend to merge with those of Drs. Ebert and Ruth, who are continuing to probe into the mechanisms of synthesis and localization of cardiac myosin and actin. The sequence of events related to the localization of myosin is particularly intriguing. At least two possible explanations have been advanced. The segregation

of the protein may be based on the translocation of cells, that is, cells containing myosin aggregate, in the heart-forming regions. Another view is that the disappearance of myosin involves the breakdown of the existing myosin and a true loss of synthesis of the protein in areas peripheral to the heart-forming regions. Transplantation studies make clear that, within the heart-forming areas, heart-

One of the first ideas suggested by the disappearance of myosin is that the analysis might be advanced by isolation experiments. For example, looking at figure 2, if a primitive-streak-stage embryo were cut into three fragments, *A*, *B*, and *C*, and each piece were grown in culture free of interaction with adjacent pieces before being analyzed for its content of myosin, a clue might be obtained as to whether the

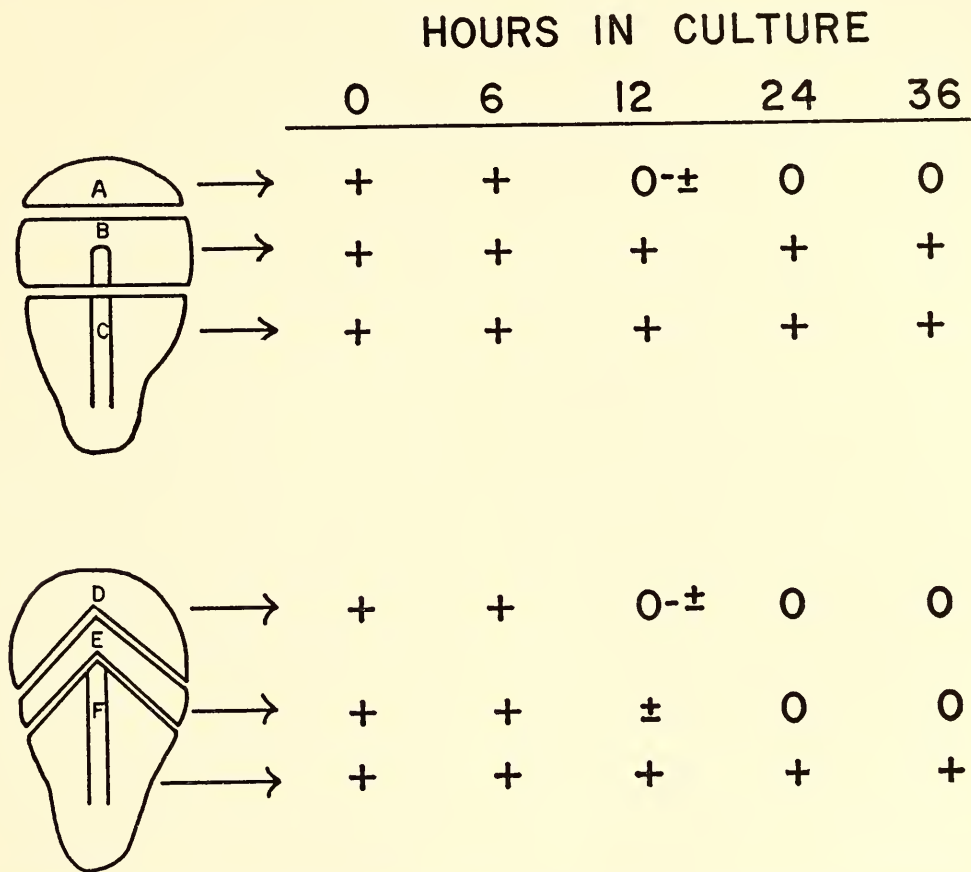


Fig. 2. Serological determination of cardiac myosin in pieces of the early chick embryo after isolation and cultivation *in vitro* for varying periods.

forming ability falls off from the center toward the periphery. In theory, it should not be difficult to distinguish whether one of these ideas is more correct than the other; embryologists often speak of the separability of morphogenesis and differentiation. Experimentally, however, dissociation of these phenomena is not always a simple task. At early stages of development, especially, the two types of mechanisms are closely interwoven. The behavior of the parts of the embryo is often unpredictable, as shown by the following experiments carried out by Dr. Ebert.

loss of cardiogenic ability and the loss of myosin synthetic ability are independent of their position with respect to adjacent tissues. The reader will recall that, in the whole embryo, section *A* exhibits the same behavior as section *C*, namely it loses its capacity to synthesize myosin. Section *B* retains its synthetic power. In isolation, however, section *A* behaves quite differently from section *C*. The posterior piece, *C*, retains its capacity to develop a pulsating heart and even after 36 hours *in vitro* retains the reactive groups of cardiac myosin. The anterior piece, *A*, does not



form pulsating masses, and it loses the reactive groups, although not immediately. The reactive groups are retained for at least 6, and occasionally for as long as 12, hours. If the embryo is cut somewhat differently, as shown in the diagram, then only the most posterior piece, *F*, retains myosin for more than 12 hours. In the absence of precise quantitative evidence, Dr. Ebert cannot say with certainty whether sections *D* and *E* differ in the time at which they lose the ability to form cardiac myosin; there is no striking difference, however.

In the opinion of Drs. Ruth and Ebert, the application of exact quantitative techniques of immunochemistry to the contractile proteins is a *sine qua non* for the elucidation of this phenomenon. Therefore, Dr. Ruth has devoted the major part of his thought and energy during the year to several basic problems bearing on the development of these techniques.

Before considering briefly his approach to the question, we should pause to re-emphasize some of the pitfalls in the use of immunochemistry, pitfalls that may lead to errors of interpretation. First, the reaction of an embryonic molecule with an antibody to a molecular species derived from an adult does not establish *identity* of the two molecules. It establishes only that there is an identity or close similarity between certain of the antigenically active groups of the two molecules. Second, the catalytically active groups of a molecule are not necessarily equivalent to the antigenically active groups. The cytotoxic or inhibitory activity of antibodies cannot be predicted from their precipitating power. Finally, immunochemical methods may indicate greater heterogeneity in a molecular population than may be meaningful to the biologist at the present time, when we have little understanding of the functionally active groups of large molecules.

These difficulties are illustrated by the immunochemical findings concerning myosin and are of particular interest from the standpoint of the development of the struc-

ture of the muscle fiber, which actually encompasses two major problems: the composition and properties of the chemical units of muscle, and their organization and integration into the discrete fiber. To what extent do the antigenic properties and physiological properties of a muscle protein derive from its precise composition, and to what extent are they the result of its position within the macromolecular complex? Thus, as Hasselbalch and Hanson and Huxley have shown, myosin can be dissolved out from whole muscle or from isolated myofibrils by procedures that remove little or no actin. Microscopic observations show that concomitant with the removal of myosin the dense anisotropic material of the so-called A band disappears, leaving the fiber almost uniformly transparent. In addition, another protein is extracted from the A band under conditions that extract myosin. Working in Dr. Albert Szent-Györgyi's laboratory at Woods Hole, George de Villafranca has shown that this extra protein is not myosin or actin. He has advanced the tentative argument that the presence of such a protein can account for the difference in density between the A (anisotropic) and I (isotropic) bands of muscle, if a continuous distribution of myosin is assumed.

At almost the same time, however, using an antimyosin serum labeled with the fluorescent dye fluorescein isocyanate, Drs. Henry Finck, Howard Holtzer, and John Marshall, at the University of Pennsylvania, have described the localization of an antigen, presumably myosin, to the A band of glycerol-soaked skeletal muscle. In addition to this demonstration, which in the writer's opinion is one of the more beautiful examples of the use of the fluorescent antibody technique, the same group also reported that muscle fibers labeled with the antibody did not contract on the addition of ATP. On the latter point, the picture is somewhat less clear in our own laboratory, for, of a total of 57 antimyosin sera tested, only 18 prevented contraction

of actomyosin filaments when ATP was added.

In spite of the extensive investigations of muscle, the relations between the biochemical and structural observations and the physiological behavior of muscle are yet to be resolved. Since much of our information on the structure of adult muscle has been obtained with the electron microscope, perhaps the most rapidly developing weapon in the armamentarium of the morphologist, it is logical to ask whether this tool has been brought to bear on the problems of cardiogenesis. Although the tissues of the early embryo are not highly favorable for electron microscopy, the first publications in the field show promise. According to Dr. Richard Hibbs, of Tulane University, the myocardium of the early chick heart consists of a network of loosely arranged cells. Between 24 and 30 hours of incubation, these cells assume a more compact arrangement. Sometime prior to 30 hours, myofilaments are formed within the cytoplasm. Some of these myofilaments are oriented to form loose bundles. At about 36 hours, the first indications of striations are visible in the region of the Z membrane, the dense line which appears to be continuous across the width of the fiber. Although contractions are noted at about 30 hours, the dense substance of the A band is laid down slowly, beginning between 48 and 60 hours of incubation. A gradual increase in density of the A band is noted. Thus, we see an apparent contradiction. On the one hand, there is no doubt that the heart begins to fibrillate as early as the second day of development; moreover, precipitin tests indicate the presence of a substance able to react with antimyosin sera even before that time. On the other hand, Hibbs states that the A band does not make its appearance until late in the second or early in the third day of incubation. Yet other evidence indicates that myosin may be localized to the A band. Conceivably, this apparent conflict of views involves little more than differences in resolving power of the

several techniques employed. Hibbs offers the suggestion that the A bands are not necessary for contraction. In earlier stages the A substance may be present although evenly distributed. Whereas there seems little doubt that the contractile system can exist in primitive form while showing the properties of contraction, the nature of the primitive components and their transformation into the complex structures of the adult remain a challenge.

The standard techniques of immunochemistry have for the most part been developed for the study of highly antigenic, stable adult proteins. The embryologist, on the contrary, prefers to select his materials for study on the basis of embryological interest, and it happens that many of the proteins with which he is concerned, e.g. hemoglobin, myosin, nucleoproteins, are either weak antigens or are unstable, or both. Therefore, Dr. Ruth has construed the application of exact immunochemical techniques as a pair of general problems, the development of a reversible adsorption technique grossly similar to zone electrophoresis and paper chromatography, and the enhancement of the antigenicity of biologically active substances. Both problems have led to the same question, how to attach a protein to another substance by unambiguous syntheses of stable covalent bonds in aqueous solution at low temperature and neutral pH. Dr. Ruth believes that at least two types of syntheses are suitable; these will be attempted as soon as the means of following the reaction rates and analyzing the products are perfected. A number of elemental and group analyses are required, since the molecular characterization of a modified protein product can hardly be considered unambiguous. Dr. Ruth has been immediately concerned with the simultaneous determination of phosphate compounds of different reactivities. Conditions have been found that permit more sensitive and direct determinations of some kinds of phosphates than have been commonly realized. A significant part of this program has re-



lied on the contribution of Dr. Jacques Mulnard, whose fundamental work on the ultraviolet absorption spectrum of phosphomolybdate has provided a phosphate method about ten times more sensitive than any used heretofore in biochemical work. Dr. Mulnard's phosphate determination, which was developed in connection with his studies of the ATPase and phosphatase activities of myosin, will be described when the work has been brought to completion.

The characterization of cardiogenesis in the chick in terms of reproducible and well defined protein fractions also awaits the application of the continuous electrophoresis apparatus. The "spontaneous" denaturation of muscle fractions on paper, as previously observed in paper strip electrophoresis for cardiac myosin, but not for skeletal myosin, poses a particularly knotty problem.

As this detailed analysis of myosin continues, exploratory investigations have been initiated in two other directions. Dr. Ebert has long been interested in the possibility of studying the development of the muscle protein tropomyosin, first described by Dr. Kenneth Bailey. Thus, when Dr. John I. White, of the Department of Physiology of the University of Maryland Medical School, proposed a joint study with Dr. Ruth of the antigenic properties of this little-known protein, which Bailey described initially as a "precursor" to myosin, it was undertaken with alacrity. The preparation of the protein, which is unusually resistant to denaturation, and its characterization (isoelectric point, sedimentation constant, etc.) are the responsibility of Dr. White; Dr. Ruth is charged with the immunochemical analysis.

Another collaborative study that can be mentioned only as in progress involves Dr. Bernard L. Strehler, of the Gerontology Section of the U. S. Public Health Service, Baltimore City Hospitals, and Dr. Ebert. Both investigators are interested in the development of a sensitive quantitative method of estimating tissue antigens, es-

pecially monovalent antigens; both are interested also in the chemistry of muscle, Dr. Ebert in its embryonic development, Dr. Strehler in changes in its properties with age. Their study, in which Dr. Strehler has carried the leading role thus far, Dr. Ebert participating only as time permits, is an effort to determine the resonant interactions of antigen and antibody labeled with different dyes (method of sensitized fluorescence).

*Metabolic characteristics of the heart-forming areas.* A comprehensive, illustrated account of the findings summarized in Year Book 55 will be available during 1957. Written by Lowell M. Duffey and James D. Ebert, this article will appear shortly in the *Journal of Embryology and Experimental Morphology*.

*The Erythrocyte-Forming Areas and the Synthesis of Hemoglobin in the Chick Embryo*

In volume 35 of the *Contributions to Embryology*, Dr. George W. Settle described the series of experiments that enabled him to map the potential hemoglobin-forming areas of the chick embryo. He concluded that the "determination" (the inadequacies of this term have been discussed earlier) of the hemoglobin-forming potentiality occurs before the appearance of the embryonic shield in the pre-primitive-streak blastoderm. Settle's study affords the basic embryological information upon which Mr. Robert G. Beard's present research is based. The erythrocyte-forming areas seem to provide an unusually favorable system for the analysis of differentiation in chemical terms. In fact, they offer one distinct advantage over the heart-forming areas, namely that the chemical nature and pathways of biogenesis of the heme moiety of the hemoglobin molecule have been studied intensively.

The aim of the research is to analyze the synthesis of hemoglobin in the early chick embryo by means of chemical and

immunochemical techniques, first paying particular attention to the site and time at which this well characterized protein can be initially detected. One of the principal questions to be considered is: are the heme and globin moieties synthesized concurrently, or does the synthesis of globin precede that of heme (as suggested by B. Thorell), or does heme synthesis precede globin synthesis? The approach adopted in this investigation should lend itself also to a collateral study of the possible effects of antiglobin sera on the ability of hemoglobin to bind oxygen or carbon monoxide.

The major portion of Mr. Beard's research was devoted to an attempt to prepare pure avian hemoglobin. The chief problem arose because the techniques previously described by others for the preparation of hemoglobin were based on the use of adult mammalian red blood cells which lack nuclei (in contrast to the nucleated cells of birds). It was necessary, therefore, to devise a technique for the removal of the nuclei of avian red blood cells together with their high content of nucleoproteins. This method, although essential to the prosecution of the research, is of somewhat less than general interest. Therefore, it will be described under the heading "Apparatus and Techniques." Although the physicochemical characterization of the preparations is yet to be completed, the initial results are in good agreement with the findings of others.

Samples of the hemoglobin preparations have been examined in the Spinco continuous-flow paper electrophoresis apparatus. Several runs were made, the best of which showed two components of low mobility under the conditions employed (Barbital buffer, pH 8.6,  $\mu=0.02$ , 400 milliamperes applied across the curtain) in a 24-hour run. Preliminary attempts to stain the electrophoresis "curtain" after a run have shown no other contaminating components. In addition, Dr. Malcolm Steinberg has been able to demonstrate two components in these samples in preliminary experiments employing the Kolin

electrophoresis apparatus for the rapid separation of differently charged molecules. Further analyses in the Tiselius apparatus and the analytical ultracentrifuge must be performed.

Samples of bovine and human hemoglobin have been prepared for use in absorption and cross-reaction studies and also as a ready source of heme for the standardization of quantitative analyses of hemoglobin solutions. The preparation of heme in quantity has not yet been successful, but prospects are good for the improvement of the available methods to suit the purposes of the study.

Preliminary studies have confirmed formation of hemoglobin in the chick embryo when grown in the well known Fell-Spratt saline-albumen medium for two days after explantation at definitive primitive streak through early somite stages. A medium of known chemical constitution that will support hemoglobin formation has not yet been found.

### *The Early Human Embryo*

Dr. C. H. Heuser, Research Associate of the Department of Embryology, returned to the Department during the summer of 1956. Having completed (with George W. Corner) for publication in volume 36 of the *Contributions to Embryology* a full description of age group x (4 to 12 somites) in the normal development of the human embryo, Dr. Heuser has focused attention on the study of embryos belonging to age group ix. In this group the neural folds are differentiating from the surrounding ectoderm to form the conspicuous paired longitudinal ridges that give rise to the brain and spinal cord. (The actual fusion of the neural folds begins in embryos one day older than those in age group ix.) The pericardial cavity and heart rudiment are appearing, and one to three pairs of somites are present.

With these criteria as a guide, the large number of specimens in the next younger



group (horizon viii) were surveyed to see whether any would qualify for horizon ix. None was found sufficiently advanced to justify changes in an earlier preliminary classification of the specimens. In the Carnegie Collection only three embryos warrant careful scrutiny as representative of horizon ix. Further study has been made of the sections and reconstructions of these embryos: models of embryo 1878 were used by N. W. Ingalls when he described the embryo; C. L. Davis used models of no. 5080 in his account of the early development of the heart. Embryo 7650, supplied by Dr. A. T. Hertig, although well prepared from the standpoint of histology, is distorted and difficult to interpret. A plastic-sheet reconstruction made subsequent to his visit to the Department of Embryology is proving to be helpful in the analysis of this specimen.

### SEX DIFFERENTIATION

In discussing embryonic sex differentiation, care must be taken to recognize that the ultimate factors concerned are to be sought in the hereditary constitution of the organism. Sex in man and all other organisms with a similar chromosomal mechanism is determined at the moment of fertilization and depends upon whether an X- or a Y-sperm unites with the egg. Before the moment of this union, the future of the egg is not fixed. Inasmuch as observable sex differentiation does not occur until later in development, it cannot be stated whether genic action immediately precedes the observable events or whether it may have taken place much earlier. A zygote of either potential male or female constitution develops first into an embryo which, morphologically, is neutral, i.e. neither male nor female in its sexual morphology. Only after the neutral stage of development has been reached does the genetic sex constitution of the embryo begin to exert a visible differential effect. In the male it is the medullary component of the gonad that becomes conspic-

Renewed attention was given to the published accounts of pertinent embryos in foreign laboratories. The one-somite embryo described by E. Ludwig is especially important. It is represented in the Carnegie Collection (no. 5982) by models and a set of serial negatives of the sections. A detailed review of this specimen is under way.

### *A Comparative Study of the Development of the Optic Primordium*

Dr. George W. Bartelmez continued to prepare for publication his comparative study of the first appearance of the optic primordium in various mammals. Evidence of proliferation of neural crest from the primary optic vesicle in the rat, pig, insectivores and primates in the Bluntschli Collection, and the macaque confirm and extend his previous findings in man.

uous, whereas the neutral gonad becomes an ovary as the result of the predominance of the cortical part. The germinal elements of the gonad, the primordial germ cells, are often recognizable well before the gonad primordium appears. Nevertheless, they are apparently not essential for the differentiation of the structural elements of the gonad. Which one of the structural components of the bisexual primordium predominates and comes into full expression is dependent upon quantitative differences in the sex-producing tendencies of the genes in the two sexes.

### *Detection of Sex of Human Embryos by the Incidence of the Sex Chromatin Body in the Nuclei of Somatic Cells*

The bisexual character of the gonadal rudiment permits experiments designed to elucidate the factors normally concerned in directing the definitive differentiation of the gonad; moreover, the occurrence of a bisexual phase of varying duration and varying sensitivity to external influences also leads to numerous "experiments of

nature" in which an imbalance of the components may result in one of several types of anomalous conditions of the genital tract. In order to understand both laboratory experiments and clinical observations, it is desirable to be able to determine the genetic sex of the embryo. Only within this decade has this become possible for the human embryo; today the genetic sex of an embryo may be determined by the study of the microscopic picture of chromatin distribution in resting cell nuclei, for cells of genetic females have a high incidence of a "sex chromatin body." This method, introduced by Dr. Murray Barr, is a particularly accurate and convenient guide to the *genetic* sex of an embryo; in errors of sex development, the clinical or social sex is often contrary to the genetic sex.

During April of 1957, Professor Emil Witschi renewed his study of sex differentiation in the human embryo, employing the technique described by Barr and associates on the embryos of the Collection, particularly those ranging in size from 10 to 20 mm. Although the embryos of the Collection were not prepared technically for chromatin studies, many lend themselves favorably to the purpose. The large round nuclei of heart mesenchyme were used for the first determinations, although the sex chromatin condition is recognizable in most well defined somatic cells. A special study is being made of the sex chromatin in germ cells of various stages. On the basis of these investigations, Dr. Witschi believes that a divergence of male and female lines of gonadal development can be recognized in 12-mm embryos (Streeter, early horizon xvii; Witschi's stage 28). A detailed illustrated report is in preparation. Of 29 embryos within the range of 2.4 and 27 mm the heart mesenchyme showed 18 times the male and 11 times the female chromatin pattern. These numbers, however, are too small to permit conclusions.

Another study initiated by Professor Witschi involves the correlations between

sex differentiation of the gonads and that of the external sex organs. This work promises to shed some light on the etiology of hypospadias and other well known types of human sex abnormalities.

### *Development of the Human Testis*

Complementing Professor Witschi's findings are the observations made by Dr. E. Roosen-Runge in the spring of 1957. His work in the Department was concerned with the problem of the early formation of the sex cords in the human testis. It has long been known that the sex cords of the rat appear suddenly and, as it were, in their entirety during the fourteenth day of gestation. The exact process by which they arise has never been clearly demonstrated. Conversely, several embryologists have stated that, in other animals, including man, the sex cords grow from the coelomic epithelium of the gonadal ridge as simple buds or sprouts and later separate from the surface and anastomose with one another to form loops. We owe much of our knowledge of the development of the human gonad to the extensive studies of Dr. Joseph Gillman, published in volume 32 of the *Contributions to Embryology*. Dr. Roosen-Runge was curious to see whether the early development of the sex cords is indeed essentially different in rat and man or whether, as the comparative embryologist might assume, they are in principle similar. For a reinvestigation of the human gonad, he used the newer specimens in the Carnegie Collection which have been stained with Mallory-azan. In these the early changes in the germinal epithelium can be followed readily, because the basement membranes are stained clearly. As in the rat, the critical stage of sex-cord formation is very brief in the human. It begins in embryos of horizon xv and is accomplished at the end of horizon xvii, a maximum span of 3 days. Although the investigation of the details of this phase of development is laborious and will take



many months to be completed, Dr. Roosen-Runge can already state that the principal loop structure characterizing the adult seminiferous tubule is established immediately as the sex cords are formed. The cords do not grow from the coelomic epithelium in a simple radial pattern but cleave off in the form of small arches in a manner still to be determined. If this concept is correct, the development of the human testis can no longer be regarded as essentially different from the rodent, and many apparent discrepancies may become reconciled. Dr. Roosen-Runge is now working on three-dimensional reconstructions of the detailed patterns in both rat and man.

*Modification and Transformation of the Embryonic Gonads of the Opossum by Treatment with Sex Hormones*

During the report year, Dr. R. K. Burns continued his analysis, already far advanced, of the transformation of the embryonic gonads of the opossum by treatment with sex hormones during the period of development. A paper entitled "Transformation du testicule embryonnaire de l'opossum en ovotestis ou en 'ovaire' sous l'action de l'hormone femelle, le dipropionate d'oestradiol" describes in detail his convincing demonstration of the phenomenon reported briefly in Year Books 53, 54, and 55 and in the *Proceedings of the National Academy of Sciences*. Inasmuch as the major conclusions of the completed aspects of this study have been included in the earlier reports, they will not be repeated. The reader interested in a full

account of this important investigation, superbly documented and illustrated, should consult the paper by Dr. Burns. At the first opportunity after his return from Paris in the spring of 1956, Dr. Burns initiated new experiments to extend these striking results, paying particular attention to such questions as the occurrence and later history of the germ cells and the fate of the surviving medullary tissue in the estradiol-transformed gonads. In addition, he carried out the first successful experiment in which the male hormone, testosterone propionate, was applied to the embryo at stage 34, the stage of development proved critical for effective action of the female hormone. Although Dr. Burns has made preliminary dissections and has begun study of the histological preparations, the results, which appear promising, are not ready for publication.

In addition, Dr. Burns continues his analysis of the rete canal system in the opossum and the collection of data on the reproductive life of this animal in Florida. Not only does the opossum provide unexcelled experimental material, but it also presents a number of intriguing problems to students of ecology and general zoology. Dr. Burns has not neglected to interest himself in the habits of this creature. In collaboration with Mrs. Burns, he published during the year one account (in *Bulletin de la société zoologique de France*) and is preparing a second paper dealing with the life and reproduction of the opossum which is scheduled to appear in a special volume of *Revue suisse de zoologie*, in honor of Dr. Kitty Ponse.

## EFFECT OF HORMONES ON CELLULAR METABOLISM IN DEVELOPMENTAL SYSTEMS

Despite the wave of optimism that was widely prevalent a decade ago, the mechanism of action of most hormones has not proved to be a simple, direct, easily recognized, and controlled action on an enzyme. In fact, there are perhaps no more than three examples of systems in which a di-

rect hormone-enzyme action has been established clearly, employing physiological levels of hormone. Substantial evidence has been presented suggesting that other hormones may act by modifying the ionic balance of the target cells, but, again, the nature of the action is not known. Ad-

mittedly, the term "physiological level" of a hormone may be inappropriate; a more correct statement may be "employing the hormone in the concentration usually found in the circulation," for little evidence is available on the effective concentration of hormone at the site of action. The interest in the Department in the role of hormones in co-ordinating developmental processes is long-standing, and it is not surprising that an effort is being made to define a system that might permit a quantitative biochemical analysis. Considerable progress has been made in Mr. Fred H. Wilt's study of chemodifferentiation of the visual pigments.

### *Chemodifferentiation of the Visual Pigments*

In the adult bullfrog, *Rana catesbiana*, as in most adult vertebrates, the visual pigment of the retinal rods is rhodopsin or visual purple, a conjugated protein containing a carotenoid pigment as its prosthetic group. The retinas of the bullfrog tadpole, however, contain a pigment that is more purple than ordinary rhodopsin, a pigment called porphyropsin by Professor George Wald, of Harvard University. At metamorphosis and transition to the land, in this and several other species, there is a sudden loss of porphyropsin, and rhodopsin becomes the predominant pigment. The onset of metamorphosis is correlated closely with the release of the thyroid hormone; therefore it occurred to Mr. Wilt that this system might provide a favorable opportunity for studying the mechanism by which a hormone effects the differentiation of a well characterized chemical system whose relation to a specific function is well understood.

Since little is known of the protein moieties (or opsins) of these two visual pigments, it is more correct to say that at metamorphosis there is a change in their carotenoids. The carotenoid conjugate of porphyropsin is retinene<sub>2</sub>, the aldehyde of vitamin A<sub>2</sub>; the conjugate of rhodopsin is

retinene<sub>1</sub>, the aldehyde of vitamin A<sub>1</sub>. The change is well defined and discrete, for vitamin A<sub>2</sub> differs from vitamin A<sub>1</sub> only by an additional carbon-carbon double bond in the  $\beta$ -ionone ring.

Mr. Wilt, a predoctoral Fellow of the National Science Foundation, is attempting to elucidate the mechanism of this change in the metamorphosing tadpoles of *R. catesbiana*. He has posited that a change in the enzymatic machinery of the organism could explain the specific changes in the photopigments. If the enzymatic change can be defined, he proposes to relate it to the metabolism of the whole organism in order to provide the basis for a further study of the transformation in relation to the onset of thyroxine release. Of course, he must establish beyond a doubt that this change actually takes place; this requirement has been met. Many possible biochemical and enzymatic changes could account in part for this phenomenon. The origin of the vitamin A<sub>2</sub> molecule should be accounted for. If both vitamin A<sub>2</sub> and vitamin A<sub>1</sub> are available to the rod, however, it may be that either the enzyme retinene reductase or the opsin molecule may change in its specificity during metamorphosis so that only porphyropsin is formed before metamorphosis and only rhodopsin afterward.

As, in general, Wilt has adapted methods described previously by other workers, they need not be fully explained here, though a few of the basic techniques should be stated in order to indicate the approach and scope of the problem. Ordinary methods of dealing with carotenoids, as discussed in Karrer and Jucker's *Carotenoids*, have been followed, although they have been scaled down to deal with microgram quantities. The Carr-Price reaction with antimony trichloride has been used to determine and differentiate vitamins A<sub>1</sub> and A<sub>2</sub>; by means of a 1-cm spectrophotometric cell that holds 1 cc, 0.25 gamma of the vitamin may be determined reproducibly in rather complex mixtures. Total



carotenoids have been extracted with diethyl ether after the tissue has been ground with sodium sulfate. Vitamin A and carotenoids are extracted from aqueous dispersions by bringing the aqueous phase to 60 per cent concentration of ethanol or methanol and extracting with petroleum ether or *n*-hexane. Retinene has been synthesized by chromatographic oxidation of vitamin A on manganese dioxide. Reduced diphosphopyridine nucleotide (DPNH) is prepared enzymatically with yeast alcohol dehydrogenase. Chromatography of carotenoids is routinely carried out on columns of alumina weakened with water or on calcium carbonate. The photopigments have been isolated by digitonin extraction of retinas after washing with buffer and an alum solution.

In an attempt to demonstrate vitamin A formation, embryonic tissue slices have been incubated with various substrates: 20 per cent Holtfreter's solution, glucose, and substrate emulsified in 1 per cent bovine serum albumin. Homogenates are incubated with magnesium, glucose, ATP, DPN or TPN, Tris and phosphate buffers (*pH* 7.2), nicotinamide, and substrate in 1 per cent bovine serum albumin. Incubations are carried out at 23° C for 1 to 2 hours. All spectrophotometric measurements have been made on a Model DU spectrophotometer or a Model DK-2 recording instrument.

*Carotenoids in the tadpole.* The amount and proportions of carotenoids in the tadpole vary somewhat, depending on the conditions in which the animals are kept and the type of diet they received before arrival at the laboratory. Therefore, exact quantitative statements are difficult to make. The amount of vitamin A<sub>2</sub> in the retina of the first-year tadpole of about 6-cm total length is about 0.08 to 0.11 gamma per retina. There is a trace of vitamin A<sub>1</sub>: 0.015 to 0.02 gamma per retina. The pigmented layers of the retina contain 0.05 to 0.1 gamma of vitamin A<sub>1</sub> and 0.13 to 0.18 gamma of vitamin A<sub>2</sub> per eye. There is also an unidentified carot-

enoid in the pigmented layer with absorption maxima at 447 and 475 millimicrons. The liver contains  $\beta$ -carotene and xanthophyll in about equal proportions; these make up most of the carotenoids present. There is also a small amount of vitamin A<sub>1</sub> but no detectable vitamin A<sub>2</sub>. Extracts of the entire gut of the tadpole contain traces of vitamin A<sub>1</sub>, xanthophyll,  $\beta$ -carotene, and an unidentified epiphasic pigment, but no vitamin A<sub>2</sub>.

*Retinene reductase.* If the larval retina could not oxidize vitamin A<sub>1</sub> to retinene, rhodopsin would not be formed even though a large excess of vitamin A<sub>1</sub> were present. If it could oxidize vitamin A<sub>2</sub>, however, porphyropsin would be formed. Wilt has shown that the larval retina definitely possesses the ability to act upon both vitamins A<sub>1</sub> and A<sub>2</sub>. For technical convenience, the experiments routinely are carried out in the opposite direction, i.e. the conversion of the retinene to the vitamin. For example, if retinene<sub>1</sub>, DPNH, and a dilute extract of larval retinas are incubated together, the retinene<sub>1</sub> is converted to vitamin A<sub>1</sub>. Similar experiments have been performed with retinene<sub>2</sub>. On a quantitative basis, one cannot say whether the enzyme is as active toward retinene<sub>1</sub> as it is toward retinene<sub>2</sub> because of the difficulty of obtaining retinene<sub>2</sub>, and no adequate means exists at present with which to compare the larval activity to adult activity with respect to the A<sub>1</sub> substrate.

*Photopigment reconstitution.* It is possible that the photopigment conversion is more complex than it seems at first sight. A change in the protein opsin might also help to explain the phenomenon. For instance, if larval opsin could not couple with retinene<sub>1</sub> but could with retinene<sub>2</sub> and the reverse occurred in the adult, some insight into the nature of the transformation might be gained. To test this possibility, porphyropsin was prepared from the retinas of tadpoles. Two very pure preparations were prepared; one from 56 retinas had its absorption maxima at 520

millimicrons (the 1-ml solution had an extinction at 520 millimicrons of 0.358). A similar preparation from 60 retinas had an extinction of 0.382 at its maximum at 520 millimicrons. Taking as the molar extinction coefficient of the preparation that of rhodopsin, 40,000, since no figure is available for porphyropsin, Wilt has calculated that there is  $1.6 \times 10^{-7}$  mole of pigment per retina, or about  $10^{16}$  molecules per retina. For the first time, to our knowledge, it has definitely been shown that the photopigment of the tadpole differs from that of the adult. Next, these preparations were bleached, and a mixture of isomers of retinene<sub>1</sub> was added in great excess. After incubation in the dark for 1 hour, hydroxylamine was added to trap the free retinene, and a difference spectrum was taken. About 60 per cent of the added retinene<sub>1</sub> contributes to the regeneration of a new pigment with an absorption maximum around 495 millimicrons. This finding shows that the larval protein can couple with retinene<sub>1</sub> to form a rhodopsin-like pigment. Under the same conditions adult rhodopsin will regenerate to the extent of about 80 per cent or a little better, indicating that there may be quantitative differences. The kinetics of formation of the new pigment from the bleached porphyropsin corresponded closely to the type of regeneration reported for pure rhodopsin preparations.

*Origin of vitamin A<sub>2</sub>.* Wilt has attempted, thus far without success, to demonstrate that the larva can form vitamin A<sub>2</sub> or a closely related substance from some precursor, but that the adult lacks such ability. If the whole larval retina or pigmented layers of the eye are incubated with various carotenoids and an energy source, some binding and possible destruction of the substrate takes place, but synthesis of vitamin A<sub>2</sub> has not been observed. Similar results were obtained with homogenates of retina. Using the conditions described above, (trans) vitamin A<sub>1</sub>, xanthophyll,  $\beta$ -carotene, and an extract of total carotenoids of embryonic liver have been

tried as substrates. Slices and homogenates of pigmented layers bind liver extract strongly, and some of the material is not extractable with chloroform; presumably, it has been metabolized to some noncarotenoid product. Preparations of retina act similarly on (trans) vitamin A<sub>1</sub>, which is strongly bound and partially destroyed after 15 minutes of incubation; liver and pigmented layers do not act upon the vitamin A<sub>1</sub>, however.

The results to date show clearly that neither differences in retinene reductase nor opsin specificity can account qualitatively for the change in photopigments. Use of pure neo-b isomers should clarify the quantitative basis of the reactions. If differences between larval and adult opsin exist, immunochemical analysis may be helpful. It is still not known how vitamin A<sub>2</sub> is formed or why it is found only in the eye. Wilt tentatively plans to approach this phase of the problem by feeding carotenoids and vitamin A labeled biosynthetically with carbon<sup>14</sup>; this approach should elucidate the precursor of vitamin A<sub>2</sub>. It is also hoped that the influence of small thyroxin pellets implanted in the eye of the premetamorphic tadpole may help determine the role of the hormonal milieu in the differentiation of the photopigments.

#### *Response of the Mesenchymal Tissues of the Guinea Pig to Estrogens*

With the help of Mrs. R. B. Moore, Dr. Ruth has devoted a part of his time to an exploration of the effect of the female sex hormones on the Foà-Kurloff Body cells in the spleen of the guinea pig. Nearly seventy years ago, Kurloff and Foà and Carbone described an azurophilic cytoplasmic inclusion in the white blood cells of the guinea pig. Since then, there have been numerous efforts to define this unusual cytoplasmic body and to delimit the conditions governing its occurrence. Dr. Ruth's interest was aroused by a number of reports suggesting that these inclusions are secretory products of the cells in which they occur in the normal guinea pig and



that they develop in an orderly pattern related to the functional development of the animal under the influence of the sex hormones. A review of existing data led Ruth to believe that he might be able to employ these Bodies, which should be amenable to chemical fractionation techniques, as experimental targets in a study of the direct action of the sex hormones. He has confirmed the earlier reports of the greater frequency of the Kurloff cells in females as compared with males, and in aged animals as compared with young animals. Cytochemical and morphological observations made in his laboratory are consistent with the recent literature.

His immediate objective is to find an amount of estrogen to stimulate the production of Foà-Kurloff Bodies in the spleen when the hormone is implanted directly in the spleen in the form of pellets; i.e. he seeks a clear-cut local action, directly on the cells, under conditions in which the more general hormonal effects are absent as judged by criteria such as disappearance of the vaginal membrane. Should such a direct effect be observed,

the research could be extended to experiments which would include studies employing tissues cultured on the chorioallantoic membrane of the chick embryo or *in vitro*. The initial results have not been encouraging. Attempts to obtain a local effect with diethylstilbestrol have failed; in view of the paradoxical effects sometimes obtained with this hormone in other experimental systems, however, its use may have been ill advised, and a second attempt was held to be justified. A study of the effects of a series of estradiol pellets is now in progress; should these experiments fail to demonstrate a direct effect, the research will be terminated. Despite the inherent interest of the problem, the writer believes that the system warrants intensive study only if the possibility of studying the control of the synthesis of the particulate body can be realized under conditions better defined than in the intact animal. In any event, the investigation has not been without profit, for it has contributed to a better understanding of the cytology and function of the spleen, in which several members of the group are keenly interested.

## THE FACTORS CO-ORDINATING GROWTH AND DIFFERENTIATION

### *Terminal Growth in the Hydroid Campanularia*

For several years, Mr. Charles R. Wyttenbach has spent each summer working at the Marine Biological Laboratory in collaboration with Dr. Sears Crowell, of Indiana University, whose interests center about problems of development in the coelenterates. This co-operative venture was continued in the summer of 1956 and again in the current year. The colonial hydroid *Campanularia flexuosa* provides unusually favorable material for the study of the rate of growth of the organism and its constituent parts in relation to their age. In approaching the problem, the investigators have studied in detail the rate of growth at the distal tip of the upright stem. The growth rate at this point determines the height of the stem. Con-

tinuous observations were made on the rate of addition of new hydranths to a single upright. At 18° C well nourished stems added 1 terminal hydranth each day for 10 days, after which the rate of hydranth addition dropped gradually over a period of days until it reached a level of 1 hydranth every 33 hours. At this point the rate remained constant over a period of 8 weeks; there was no evidence of a further continuous slowing, and Crowell and Wyttenbach believe that given the correct nutritive conditions terminal growth might go on indefinitely. These conditions are never met in nature, where an upright with more than 15 to 20 hydranths is seldom found.

In further experiments, the rate of terminal hydranth production was tested for several levels of the stem. In this phase

of the study, either the distal portion of the stem was removed and the growth potential of the stump tested, or sections of the stem were studied in isolation. It was found that, as a general rule, the older the level of the stem, the slower the rate. In tall, old stems, however, there is a level some distance behind the tip (about 15 nodes below the terminal hydranth) which is the slowest; below it all levels are similar. Over-all, the results support the argument that the rate of hydranth production is related to the age of the tissues involved and that the age of the whole stem affects the rate in the terminal region, a region which itself consists of young tissues. Not all the evidence can be explained on this basis; further work is in progress.

#### *Organ-Specific Regulation of Growth and Differentiation*

One of the objectives of research in the Department at this time is to examine intensively the idea that the co-ordination and regulation of developmental processes presuppose the generation in each cell strain of paired compounds of complementary configuration and that differentiation and growth of a cell strain are governed by a balance of cell-type-specific molecules. In the introduction to this topic in Year Book 55, the writer pointed out that, although several lines of evidence have established the working hypothesis of selective chemical co-ordination among cells of identical type by direct exchange of type-specific compounds, research has only begun on the nature of the mechanisms involved. During the year, progress has been made toward bringing these problems into sharper focus.

The transplantation of adult cells to an adult organism of a different genetic make-up commonly results in an immune reaction, leading to the death of the implanted materials. The contributions of many, especially Professor P. B. Medawar and his co-workers, have made clear that the individual-specific antigens (probably

of nuclear origin, although this point has not been resolved fully) of the donor cells evoke in the host the production of antibodies that ultimately destroy the donor cells. The precise nature of the immune reaction has not been clarified; several facts suggest that it must be mediated by the cells of the host, e.g. passive immunization has been achieved only through transfer of intact lymphoid cells, and tissue homografts survive when grown inside chambers whose walls (filters) permit the passage of proteins but exclude cells.

When adult cells are transplanted to an embryo, however, the transplant survives throughout most or all of embryonic life and occasionally persists after birth. From the studies of Billingham and Medawar and their associates we know that the exposure of embryos in the latter part of the developmental period, and of newborn animals, to adult cells results in a state of tolerance, for, when these animals are challenged with the homologous antigens in later life, they are incapable of producing an immune reaction. The mechanism for the production of antibodies by the embryo develops only in the late embryonic stages and in the neonatal period, and during this period it is subject to modification.

When adult cells are transplanted to the early embryo, well in advance of the critical period for the induction of tolerance, however, another consequence of fundamental importance is observed, namely a highly significant stimulation in the growth of the homologous tissue. A case in point is the stimulation of growth of the embryonic chick spleen following transplantation of a fragment of adult chicken spleen to the chorioallantoic membrane of the 9-day host embryo. Dr. Ebert's studies, which stemmed from the pioneering observations of Danchakoff, Willier, and others, have shown the reaction to be quantitatively organ-specific; i.e., of eleven donor tissues studied, only three are capable of evoking the response; these are,



in order of decreasing effectiveness, spleen > thymus > liver. The reaction is class-specific but not species-specific, adult mammalian spleen having been shown to be ineffective, whereas fragments of spleen of other avian species do stimulate the growth of the chick spleen although never to the same extent as the homologous tissue. Earlier observations showed also that the age of the donor animal is a critical consideration. The effective factor or factors are absent from the embryonic spleen during the first two-thirds of the developmental period, making their appearance first at about 14 days of incubation. Hence grafts of embryonic spleen from donors younger than 14 days are ineffective. The effective factors are accumulated slowly, reaching the "adult" level by 6 weeks post-hatching. In immunochemical studies, although Ebert was able to demonstrate the appearance of at least three specific splenic antigens at about the fourteenth day of incubation, he did not succeed in establishing a causal relation between their appearance and the onset of specific stimulatory capacity.

The next major step in the analysis was based on the use of radioactively labeled transplants; these studies showed distinctly that, when the proteins of the donor tissue, like kidney or spleen, were radioactively tagged by injecting into the intact animal  $S^{35}$  methionine in quantities sufficient to ensure maximal labeling of the tissue proteins without radiation damage, labeled materials from the graft were localized predominantly in the homologous organ of the host. The tentative hypothesis was advanced that macromolecular components released by the graft were localized selectively in the homologous organ; Dr. Ebert proposed that, although these macromolecules were not incorporated directly into the host proteins, the amino acids and peptides into which they were fragmented before incorporation never entered the general amino acid pool.

The results of this phase of the investigation, which included cytologic and auto-

radiographic studies and analyses of deoxyribonucleic acid in stimulated tissues, clearly demonstrated that the stimulation did not result from a massive transfer of intact cells. On the other hand, they did not rule out the transfer of a seed population of viable cells, a possibility made attractive by the rapidly developing studies demonstrating the successful transplantation of bone marrow and other sources of prospective blood cells (hematoblasts). Several considerations argue against the importance of the transfer of viable cells in mediating the growth stimulation, for example: the observed difference in effectiveness between embryonic and adult grafts, whereas such a difference has not been observed in hematoblastic seeding (in fact, it has been argued, the more potential hematoblasts, the better); the absence of plasma cells, characteristic of the adult, in the affected host tissues; and the experiments of others, including Teir and Weiss, which indicate the stimulatory effectiveness of frozen, thawed triturated tissues. In fact, devitalization often has enhanced specificity and stimulatory effectiveness. Nevertheless, the requirement for intact cells has not been ruled out completely. Obviously a number of questions remain to be answered. During the year several new observations were made; they are summarized in the following paragraphs.

*Cytologic analysis of the development of the normal and stimulated spleen.* It is unusual to find, at this stage in the development of the field of embryology, that the normal ontogenesis of an organ or tissue is not sufficiently well described and understood to serve as a solid foundation for a critical interpretation of experimental findings. As this investigation progressed, however, Dr. Ebert became more and more aware of the fact that a clear statement of the normal progression of cell types and changing tissue architecture, commonly available for most tissues, was not available for the avian spleen. Dr. L. E. DeLanney, of Wabash College, who has shared Dr. Ebert's interest in the problem

for several years, was invited to collaborate in an intensive study of the normal and experimental spleens. In this joint undertaking, the workers have available for analysis several thousand cytological and autoradiographic preparations of normal tissues and graft and host spleens. Their study, not yet completed, will be presented in a future volume of the *Contributions to Embryology*.

In the chick the primordium of the spleen is observed first at stage 23 of the Hamburger-Hamilton series (about  $3\frac{1}{2}$  days) as a dome of mesenchyme immediately dorsal to the region of the stomach and duodenum. Although splenic sinuses and the beginnings of the splenic vein are visible on the fourth day of incubation, little tissue differentiation is seen. This dome of generalized mesenchyme persists until stage 25 ( $4\frac{1}{2}$  days), when differential growth and other morphogenetic processes result in a shifting of the spleen to the mesentery as an isolated mass. The first distinct cellular differentiation is observed at approximately stage 35 (8 to 9 days), at which time granuloblasts, recognizable by their thin basophilic cytoplasm, large clear nuclei, and large, acidophilic nucleoli can be identified. Granulopoiesis continues as the primary activity through stage 37 (the eleventh day), when the arterial supply is observed. During all this time mitotic activity continues unabated. The first signs of tissue organization are noted at stage 40 (14 days), and by the seventeenth day (stage 43) the follicles with central arteries, and the granulocytic interfollicular pulp, are relatively distinct, this condition becoming more and more pronounced in the hatching and post-hatching periods.

The question arises next whether this sequence of events is altered when the growth of the spleen is stimulated by the homologous graft. It is not altered significantly during the period from the tenth through the seventeenth days. The growth of the spleen results from a slight increase in cell size and a pronounced increase in

cell number. The pattern of cellular differentiation and morphogenesis in the experimental spleens during this period is no more varied than that in the series of normal spleens. The investigators find no evidence of massive cell transfer; moreover, if seed populations of cells are localized in the spleen, they must be incorporated into the normal architecture of the spleen; of course, one might expect, from studies by Moscona and others of dissociation and reassociation of embryonic tissues *in vitro*, that such would be the case. However, cell types characteristic of the adult, e.g. plasma cells, have not been observed. In a small, but important, proportion of rapidly growing stimulated spleens, a distinct, characteristic change is noted beginning on the eighteenth day of incubation. This significant departure from the normal has been observed most frequently in embryos in which the transplanted spleen was implanted somewhat earlier than usual, i.e. on the seventh rather than the ninth day. The cellular nature of this phenomenon and the conditions resulting in its expression lead the investigators to postulate that shortly before hatching the transplanted adult spleen produces an immune response directed against the host. This hypothesis will shortly be treated more fully.

*Growth stimulation in the decapitated chick.* In a recent discussion of phases in embryonic development, Professor B. H. Willier emphasized that the main features peculiar to the different periods of development can be characterized: he described the period in the chick between the tenth and thirteenth days of development as the time of functional integration, the period in which the humoral connections between organs via the blood-vascular system seem to make their appearance. During this period the embryonic pituitary gland begins to function and exert influence on the so-called target organs; the evidence supporting this argument need not be reviewed here. It will suffice to point out, first, that the ability of the embryonic spleen to act



in promoting the growth of the homologous organ when transplanted arises first just at the close of this period of functional integration; and, second, that Dr. Norman W. Vogel has demonstrated recently that when the embryo is hypophysectomized the synthesis of cholesterol and the growth of the embryonic spleen are modified beginning at 12 to 14 days. Therefore, it seemed imperative to determine whether the hypophysis was, indeed, an intermediary in the stimulation of splenic growth by splenic grafts.

Experiments carried out by Dr. Ebert and Miss C. Coffman have shown that the reaction proceeds without modification or reduction in the absence of the hypophysis. The technique and results do not necessitate detailed description at this time. During the second day of development, the embryo is partially decapitated by the simple expedient of severing the anterior brain region, including the hypophysis, as shown in figure 3. Although the mortality resulting from this operation, originally described by Fugo, and modified by Vogel and others, is high, many embryos survive within the shell, even beyond the expected time of hatching, a feat which they cannot accomplish. The experimental cases are compared with several types of controls, including animals in which an eye cup is removed, as shown also in figure 3. Figure 4, plate 2, permits a comparison of normal and partially decapitated embryos at 14 and 17 days.

Next, a second step, chorioallantoic transplantation, is superimposed on the first experimental technique; after decapitation, embryos are permitted to develop until the ninth day, at which time a graft of adult spleen or another adult tissue is made. The embryos are reincubated and allowed to develop for 5 to 9 additional days, when they are recovered for study. The results confirm fully the finding of Vogel that the spleen grows slightly more rapidly in the decapitated chick than in the normal, and show clearly, moreover, that the spleen of the decapitated embryo responds equally

as well as the spleen of the normal embryo to an homologous transplant. These results are of interest in another connection: Kaliss and co-workers at the Jackson Memorial Laboratory have described a phe-

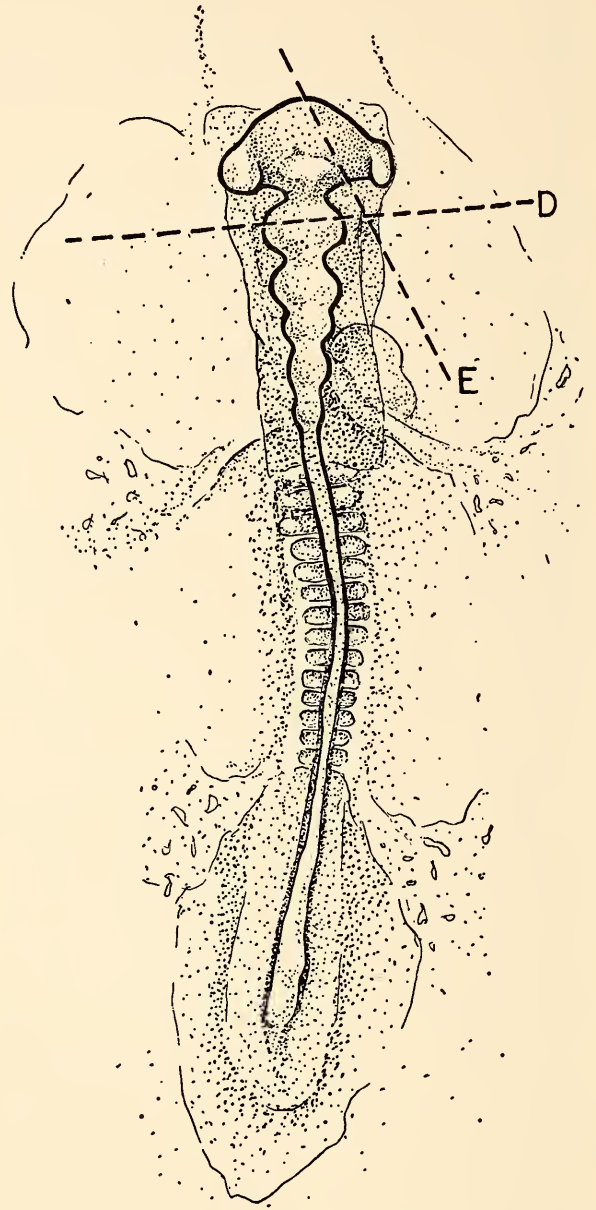


Fig. 3. Chick embryo at stage 11+ (38-40 hours), showing levels at which cuts are made for partial decapitation (D) and removal of an eye cup (E).

nomenon, known as the enhancement reaction, in which the administration to mice of extracts of certain tumors results in the enhancement of growth of those tumors. Although the nature of the enhancement reaction is unknown, it does appear to be mediated in some manner by an immune



Fig. 1. Cardia bifida in the chick embryo, produced by placing a crystal of acetylcholine on the endoderm of the area pellucida 24 hours earlier.





Fig. 4. Partially decapitated and normal chick embryos; upper pair, 14 days; lower pair, 17 days.

mechanism, which can be modified in turn by modifying the pituitary-adrenal axis. Although it is generally stated that the embryo is incapable of forming antibodies, we know from the demonstration of acquired tolerance that the antibody-forming system can be altered during development; hence the possibility that the splenic growth phenomenon might be mediated by an immune mechanism, of a type hitherto undescribed, cannot be discounted. Although the experiments just reported do not provide convincing evidence of the dispensability of such an immune mechanism, they do provide additional indirect evidence for it, inasmuch as grafts are effective in embryos in which the adrenal has been appreciably modified in the absence of the pituitary.

*Localization of injected subcellular fractions.* During the year Dr. Ebert completed the analysis of a substantial body of data collected in previously unreported experiments, some of which were carried out in collaboration with Dr. A. M. Mun. By way of background, several experimenters have suggested that not only are devitalized tissues effective in promoting homologous organ growth, but also that some devitalized breis show an augmented specificity and stimulatory effect. For several years, Dr. Ebert has been engaged in extending his own study in an effort to define more precisely the nature of the substances transported from graft to host tissue and the manner in which they act. The approach has been a straightforward attempt, first, to determine whether radioactively labeled nonviable homogenates of organs injected into the embryo via the vascular system tend to localize differentially in the homologous organs. Having established this, he proceeded to the second objective, determining whether all the constituents of the homogenate behaved in the same manner or whether only certain fractions showed a predominant localization in the homologous organ.

The experiments can be summarized briefly. Adult tissues were labeled, as in

earlier experiments, by the injection of methionine labeled with sulfur<sup>35</sup>. These organs were stored in the frozen state, after which they were homogenized, and a small amount of the homogenate was injected directly into the chorioallantoic vessels of the 9-day embryo. Parenthetically, it was learned quite early that predominant localization does not result from injection by way of the yolk sac, and so intravascular injection is necessary. Moreover, by the latter route the embryo will tolerate no more than 0.1 ml of the homogenate; several factors, including the absence of a clotting mechanism, result in a relatively low frequency of successful operations. The results of these experiments, in which labeled kidney and spleen homogenates were injected on either the ninth or tenth day of incubation with recovery after 24 hours, are given in tables 1 and 2. They show that the exposure of the embryo to a very small quantity of tissue homogenate for only 24 hours results in a differential incorporation of the label in the homologous organ. The differential is not of the same order as that obtained with living transplants, acting over several days, but it is consistent and reproducible.

The next step was the fractionation of the homogenate following the techniques described by others, especially Claude, Hogeboom, and co-workers, and Zamecnik and co-workers, in which nuclear, mitochondrial, microsomal, and "supernatant" fractions are separated according to their pattern of sedimentation in the centrifuge. The annual report is not an appropriate place to discuss fully the merits and pitfalls of this approach. These techniques do not permit a high degree of precision; they are experiments of a rough and ready kind, but the writer believes that they can be extraordinarily useful provided that the limitations in definition and resolution are kept constantly in mind.

The data presented in table 3 illustrate the selective localization only of the microsomal and supernatant fractions, and not



TABLE 1. Localization of Radioactively Tagged Kidney Homogenate

	Injection Day 9 Recovery Day 10				Injection Day 10 Recovery Day 11	
Counts per minute injected per embryo..	604		604		720	
Mg injected per egg.....	0.1		0.1		0.1	
No. embryos injected.....	84		71		70	
Organ studied .....	Kidney	Spleen	Kidney	Spleen	Kidney	Spleen
No. organs analyzed.....	30	30	25	25	23	23
Dry weight, mg.....	29.1	20.6	27.0	19.1	31.3	21.4
Weight counted .....	20.8	20.6	20.3	19.1	21.6	21.4
Corrected cpm per 20 mg.....	54	42	50	42	69	56
Relative specific activity.....	1.00	0.77	1.00	0.84	1.00	0.81

TABLE 2. Localization of Radioactively Tagged Spleen Homogenate

	Injection Day 9 Recovery Day 10				Injection Day 10 Recovery Day 11	
Counts per minute injected per embryo..	1460		1460		1802	
Mg injected per egg.....	0.1		0.1		0.1	
No. embryos injected.....	75		75		60	
Organ studied .....	Kidney	Spleen	Kidney	Spleen	Kidney	Spleen
No. organs analyzed.....	24	24	27	27	19	19
Dry weight, mg.....	25.9	20.1	29.0	21.4	23.6	18.7
Weight counted .....	25.9	20.1	20.3	21.4	20.7	18.7
Corrected cpm per 20 mg.....	165	190	160	201	232	283
Relative specific activity.....	0.87	1.00	0.80	1.00	0.82	1.00

TABLE 3. Relative Specific Activities of Host Tissues following Injection of Tagged Organ Fractions

Injection Day 9, Recovery Day 10

Experi- ment No.	Host Tissue	Kidney Fraction Injected			Supernate
		Nuclear, 10,200 g, 30 min	Mitochondrial, 36,000 g, 30 min	Microsomal, 144,000 g, 60 min	
1	Spleen	1.05	1.00	0.49	0.61
	Kidney	1.00	1.00	1.00	1.00
2	Spleen	1.02	0.97	0.53	0.76
	Kidney	1.00	1.00	1.00	1.00
3	Spleen	1.04	0.94	0.56	0.69
	Kidney	1.00	1.00	1.00	1.00
4	Spleen	0.98	0.01	0.52	0.70
	Kidney	1.00	1.00	1.00	1.00
Spleen Fraction Injected					
1	Spleen	1.00	1.00	1.00	1.00
	Kidney	0.97	1.04	0.44	0.80
2	Spleen	1.00	1.00	1.00	1.00
	Kidney	0.96	0.95	0.61	0.84

of the nuclear and mitochondrial fractions. The properties of the microsomal and supernatant fractions that determine the specificity, or are essential to the localization, are nondialyzable, and are destroyed by heating at 80° C for 5 minutes, and by ashing. We are not prepared to discuss the chemical properties further at this time. Nor does it seem wise to speculate broadly on the significance of the findings. The possible implications are apparent to all who are familiar with the importance of the interaction of the enzymes and ribonucleic acid of the supernatant fraction and the ribonucleoprotein of the microsomes in the incorporation of amino acids and synthesis of protein. Yet, Dr. Ebert has emphasized that the experiments have one shortcoming: they deal only with one aspect of the problem, namely selective localization. They do not necessarily contribute to an understanding of why the homologous organ is stimulated. The small quantities of subcellular fractions tolerated by the embryo do not result in a significant growth stimulation. It appears that a continuing supply of injected materials may be necessary in order to bridge the gap between the two types of experiment. This we have thus far failed to achieve; experiments to this end will be continued.

*Serial transfer of growth-promoting activity.* During the year, Miss Coffman and Dr. Ebert made an unexpected observation, which is of such interest that it must be reported at this time, even though little more than a tentative hypothesis can be advanced in explaining the results. The experiments were started as the result of an observation made in experiments first carried out by Dr. Robert A. Tolman at the suggestion of Dr. Ebert. The objective of these earlier experiments was to determine the effect of grafts of adult spleen made to the coelom of the host embryo at 3 days of incubation, a time at which the host spleen has not yet appeared. According to Dr. S. M. Rose, such an experiment should result in the suppression of splenic development. It did not; on the contrary, the usual stimu-

lation was observed. In fact, the developing spleen of the host was stimulated greatly by the tenth day, and the question was raised whether such a stimulated 10-day embryonic spleen might now in itself be an effective donor (normally the 10-day spleen is ineffective).

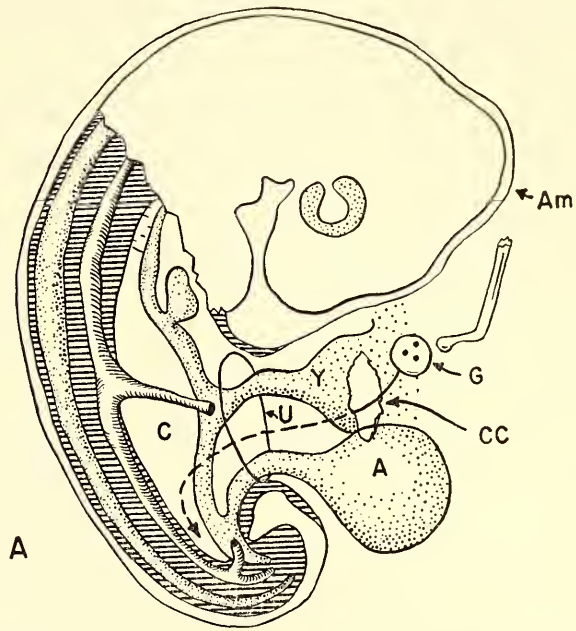


Fig. 5. Diagram of 4-day chick embryo showing insertion of intracoelomic graft.

TABLE 4. Effects of Intracoelomic Grafts of Chicken Spleen

Age of Donor	No. Cases	Weight of Host Spleen at 10 Days, mg	Range, mg
None .....	37	3.9	2.6- 5.1
Adult chicken ...	44	22.4	4.9-43.0
Chick, 1-7 days			
post-hatching ..	17	9.1	3.1-16.7
16-day embryo ..	22	5.3	2.9- 8.8
10-day embryo ..	30	3.7	2.4- 5.4

This idea has been tested, largely through the efforts of Miss Coffman. The experiment may be outlined briefly; consult figure 5 and tables 4 and 5. To set the stage, a number of grafts of spleen from embryonic, juvenile, and adult donors were made to the coelom of the 4-day chick embryo, following a method described by Dossel. The findings confirm the author's earlier statements concerning



the importance of the age of the donor. Utilizing the data from the preceding experiments as a base line, grafts were made to a number of 4-day embryos, which were permitted to develop until the tenth day, when the host spleens were harvested and used as the donor tissue for a second set of grafts, and so on. The experiment was terminated after the fourth set of grafts was recovered.

in the splenic primordium, whereas embryonic spleen cells proliferate in the graft but do not stimulate the host spleen? Mitchison has reported interesting experiments indicating that, as a result of implanting adult turkey spleen into the chick embryo, the host's red blood cells are true chimeras of chicken and turkey antigens. It should be emphasized that reference is not made to simple erythrocyte mosaicism,

TABLE 5. Serial Transfer of Growth-Promoting Activity

I	II	III	IV
Original graft 0.5-1.0 mg ACS	10-day host spleen 22.4 mg	10-day host spleen 14.1 mg	10-day host spleen 12.9 mg
	Use 0.5-1.0 mg as donor tissue	Use 0.5-1.0 mg as donor tissue	Use 0.5-1.0 mg as donor tissue
			10-day host spleen 19.7 mg

As can be seen from inspection of table 5, the growth-promoting activity is not diluted by serial passage, but is maintained relatively constant, considering the qualitative nature of the experimental approach. At first thought, the simplest and most straightforward explanation of these observations is the colonization of the spleen by whole cells capable of reproduction. The writer has always favored the adoption of the most economical explanation; yet the colonization of whole cells is not a fully satisfying argument; the question remains: why should adult cells, which do not proliferate in the graft, proliferate extensively

in which two different populations of cells are present, but rather to true chimerism, in which each cell is a composite of the two types of antigen. In view of the several lines of evidence discussed, the tentative hypothesis of an exchange of sub-cellular or molecular constituents appears justified. In fact, Dr. Ebert has advanced two alternative proposals: first, that the growth of the homologous organ is evoked directly by such a transfer; second, that the growth of the homologous organ is the result of a dual process requiring the colonization of the spleen by living adult cells, which, as the result of the acquisition

of constituents transferred from the host cells, now acquire new properties enabling rapid growth.

To design a single experiment to resolve the question is difficult; apparently, however, one approach should be particularly revealing, namely the encasement of grafts in cellulose ester membrane filters, which will permit molecular or particulate transfer but not cellular colonization. Technical difficulties impeded these experiments for some time, but recent observations by Dr. Jacques Mulnard and Dr. Ebert (in consultation with Dr. Clifford Grobstein) point to the resolution of the obstacles.

*An immune reaction: graft against the host.* Stemming from the studies of Jacobson at the University of Chicago, who showed that the lethal effects of whole-body irradiation could be prevented by shielding the spleen of the animal, a series of studies by several groups has dealt with the radiation-protective effects of extracts and homogenates of bone marrow and spleen. These investigations have branched and followed two trails, one of which has led to the tentative conclusion that under many conditions protection or recovery can be achieved with nonliving extracts provided that the host's hematoblasts are not totally destroyed. The other trail has led to the finding that the bone marrow of the recipient animal may be "reseeded" by hematopoietic cells of a donor animal, a finding of significance and basic interest. It has led, however, to demands for the immediate establishment of a system of "marrow banks" for treatment of radiation cases and other projected clinical applications, for example the treatment of hypogammaglobulinaemia (in which patients are unable to make antibodies). These are worthwhile goals, toward which research must be continued; in recent discussions with clinically minded associates and in several lectures, however, the writer has sounded a note of caution against their premature application to man. Further research may prove this pessimistic note unwarranted.

The facts are these. Seeding of the bone marrow of irradiated animals does permit recovery from the effects of radiation. Lost sight of, however, is the relatively high frequency of unexplained deaths occurring after recovery from exposure to irradiation. In recent months, independent observations in three laboratories suggest that adult blood cells transferred to a late embryo, newborn, or juvenile host may produce an immune reaction *against the host*, which in some cases may cause its death. Though all these observations are clear-cut, this tentative explanation cannot be regarded as totally convincing; the crucial experiments have not been done. For example, in attempting to induce tolerance by injecting newborn mice with spleen cells from donors of different strains, Billingham and Brent have found that in a number of interstrain combinations only partial tolerance is produced, accompanied by a number of specific deficiencies. In other combinations, these deficiencies are more pronounced, leading to the death of the recipient. Characteristically, the lymphoid tissues of these animals are grossly aberrant: the lymph nodes are lacking, the spleens fibrotic and deficient in Malpighian corpuscles; even Peyer's patches are missing. Billingham and Brent believe the explanation to be that suggested above: the donor spleen cells produce antibodies against the host; for reasons not yet clear (although several possibilities may be proposed), the primary reaction is against the host lymphoid system. Comparable experiments and conclusions have been reported for the chick (18-day embryos to recently hatched animals) by Simonsen in Copenhagen. DeLanney and Ebert have made similar observations both in the chick and in the larval salamander *Taricha torosa*. As described earlier, chorioallantoic grafts of adult spleen result in the growth of the homologous organ; when grafts are allowed to continue beyond the seventeenth day, however, a small but consistent fraction of the host spleens shows a striking degenerative change characterized by the



destruction or modification of the endothelium and vascular bed, which leads to a stasis in flow of granulocytes, their accumulation in cystic masses, and eventual death. The cystic condition of the spleen occurs with a frequency approximating that of the death of chicks at or shortly after hatching. The belief that the primary locus of action of the antibody produced by the graft is the vascular bed is strengthened by the observations of others, including Mellors and Pressman, who have found that frequently antiorgan sera labeled with radioisotopes or fluorescent dyes are localized first in the vascular bed of the target organ.

Finally, DeLanney's observations on the salamander may be described briefly. Grafts of adult salamander spleen have been made either into the coelom or into a pocket in the dorsal fin of the larval salamander. Although there are differences in the behavior of the transplanted tissues in the two sites, grafts in both sites lead not to growth stimulation but to suppression of the host spleen. The analysis is not yet sufficiently advanced to permit further discussion, but for the present Dr. DeLanney has taken as his working hypothesis a "graft against the host" immune mechanism.

#### *Induced Aspermatogenesis in the Guinea Pig*

Year Book 55 contained the statement that, in consultation with Dr. David W. Bishop, Dr. Seymour Katsh had initiated a program of investigation designed to explore further the extraordinarily interesting demonstration by Freund and his associates that the injection into the male guinea pig of adjuvants combined with autologous and homologous testicular homogenates induced a condition of aspermatogenesis. The investigation has been prosecuted vigorously, and a number of new and thought-provoking observations can be reported.

#### *The effects of injections of homologous*

*testicular homogenate combined with adjuvant upon the testes of guinea pigs.* Between 58 and 135 days after the intracutaneous injection into adult male guinea pigs of an emulsion of guinea pig testis and complete adjuvant (paraffin oil, Arlacel oil, and dead *Mycobacterium butyricum*), the spermatogenic tissue of the recipient animals was found to be damaged. The degree of damage varied from mild to severe and appeared to be a function of time as well as of the sensitivity of the individual animal. The accessory organs of reproduction were unaffected by the treatment.

It has been argued that damage to the spermatogenic tissue is the result of an immune reaction, which may operate in the following manner. The preparation of the testicular homogenate and its combination with adjuvant result in a change in its properties sufficient to enable it to act as an antigen in the homologous species. (The concept of individual-specific antigenicity cannot readily be invoked here.) The antibodies produced by the host, however, are not sufficiently specific to distinguish between normal and slightly altered testicular antigens; hence the animal's own testis is destroyed. The work of Freund and the current studies have disclosed that sperm-immobilizing, sperm-agglutinating, and complement-fixing antibodies appear in the sera of the injected animals and not in the sera of control animals injected with adjuvant alone. Yet there are reasons for believing that these true circulating antibodies may not be instrumental in inducing aspermatogenesis. For example, Katsh and others have been unable to demonstrate a correlation between antibody content and aspermatogenic effectiveness. Moreover, although other animals like the rabbit are highly effective in producing circulating antibodies of this type, aspermatogenesis can be induced only with difficulty. These considerations led to investigations of other types of immune reactions, resulting in the finding that the ileum of the sensitized guinea pig injected with homologous testis or sperm responds

*in vitro* to a challenging dose of guinea pig sperm by contracting. This reaction suggests an allergic response of the anaphylactoid type. Work in this direction is continuing. Dr. Katsh has not yet undertaken experiments to determine whether aspermatogenic activity can be transferred passively; that passive transfer could not be achieved by the transfusion of the serum of treated animals might be predicted, but whether a massive transfer of cells from the lymph nodes draining the site of injection might succeed is another question.

*Effects of injections of homologous brain and heterologous testicular homogenates on the testes of guinea pigs.* If aspermatogenesis is elicited by a primary antigenic stimulus, the spermatogenic tissue might be affected by injecting antigens that cross-react with testicular antigens. Reports in the literature indicate that brain and testicles share a common antigen and that the testes of different species may exhibit cross reactivity. Therefore, homologous brain and heterologous testicular tissue were injected in combination with adjuvant. The experiments proved the working hypothesis correct, for guinea pig brain and testicular tissue from roosters and monkeys as well as human semen induced spermatogenic lesions in the guinea pig.

*Effects of injections of damaged guinea pig testes plus adjuvant upon the testes of guinea pigs.* Another test of the immune nature of the injury was made based on the following argument: if the damage is so specific as to injure the germinal portions of the testes selectively, then the antigenic material should be found only in the spermatogenic tissue. This test was established in the following experiment. Guinea pig testicles severely depleted of spermatogenic tissue as a result of injections of homologous testis were homogenized and injected with adjuvant into other guinea pigs. No significant effect on spermatogenesis was obtained by the injections of damaged testes. Therefore, Katsh has demonstrated that the antigen is found only in the spermatogenic tissue.

This experiment also establishes that androgens present in the testicular homogenates could not have caused the spermatogenic lesions.

Further experiments are in progress to localize, if not define, the precise stage(s) of spermatogenesis at which the antigen is present. The results upon which all the conclusions described in the foregoing section are based will appear shortly in a paper by Drs. Katsh and Bishop in the *Journal of Embryology and Experimental Morphology*.

*Aspermatogenesis in other species.* Attempts have been made to induce aspermatogenesis in rats, mice, and rabbits by injections of homologous and heterologous testis homogenates plus adjuvant. Mild damage (compared with the guinea pig) has been obtained in the rat. The rabbit and mouse appear to be resistant to this type of treatment. But when rabbits were injected 5 times during 10 days intravenously with saline suspensions of rabbit ejaculate and then sacrificed on the seventeenth to eighteenth day after the first injection, destruction of spermatogenic tissue was found. Other rabbits treated in the same manner but sacrificed earlier did not show injury. This result suggests that the timing of the injections may be critical and points to a possible allergic response. Further exploration is required to establish this important point. If the result in the rabbit can be shown to be anaphylactinogenic, a parallel with the guinea pig may be established.

*Chemical nature of the antigen.* Other work in progress relates to the chemical nature of the antigen and to the contribution made by the adjuvant relative to aspermatogenesis. Since aspermatogenesis can be induced by this procedure only by emulsifying the antigen together with the adjuvant and injecting the emulsion, it has been thought that the adjuvant served as a storage depot permitting a slow release of antigen over a long period, and thus effecting a potentiation of the immune reaction. This may be true, but the



adjuvant may also contain some specific factor that is a *sine qua non* for the production of aspermatogenesis: if an emulsion of testis using an incomplete adjuvant (only paraffin oil and Arlacel A) is injected, no testis damage is noted. The presence of either *Mycobacterium tuberculosis* or of *M. butyricum* is required. Therefore, the bacteria apparently make a contribution. Since both these organisms are acid-fast, Dr. Katsh thinks that the condition described as "acid-fastness" represents an important component. Adjuvants containing nonacid-fast bacteria have been prepared, and so far it has been determined that adjuvant with *Salmonella typhimurium* (a nonacid-fast bacterium) is incapable (when emulsified with testis) of producing aspermatogenesis. Furthermore, a lipopolysaccharide extracted from tubercle bacilli by Dr. J. Asselineau (Paris) (and kindly provided by him) was found to replace the *M. tuberculosis* or *butyricum* in the adjuvant and to produce testicular damage. When this same lipopolysaccharide is added to the *S. typhimurium* adjuvant, aspermatogenesis is obtained. Various other crystalline compounds from tubercle bacilli obtained from many different sources have no effect. These findings suggest that a specific chemical isolated from acid-fast bacteria is involved in the production of experimentally induced aspermatogenesis.

Similar lines of reasoning have been applied to the chemical nature of the antigen. The current working hypothesis is that a lipopolysaccharide extractable from testis combines with the lipopolysaccharide from acid-fast bacteria to induce testicular damage. Work along these lines is being continued.

*Injection of homologous and heterologous extracts into the female.* A large number and variety of exploratory experiments have been carried out with two principal goals in mind: the possible induction of infertility in the female, and the alteration of the differentiation or

growth of developing tissues of the fetal guinea pig *in utero*. Additional interest in the latter experiments has been stimulated by the recent report of Dr. Salome Gluecksohn-Waelsch that in mice the formation of the developing nervous system can be altered by the injection of brain extracts into the pregnant female. Although many of these experiments have not yet reached the point at which they can be discussed with profit, mention of the following preliminary findings appears to be justified.

Groups of female rats were injected with adjuvant alone or in combination with homologous ovary, testis, adrenal, prostate, seminal vesicle, thymus, or thyroid. The organs of the offspring were not affected. Female rabbits were injected with homologous ovary, testis, corpora lutea, and endometrium (each with adjuvant), or with adjuvant alone. A high percentage of offspring of the animals injected with ovarian and endometrial preparations were born dead or died shortly after birth. Two of 5 females injected with testis failed to conceive. All females had previously been tested for fecundity and viability of offspring. Female guinea pigs were injected with adjuvant alone or in combination with homogenates of homologous prostate and seminal vesicle without obvious effect. Curious results were obtained, however, when female guinea pigs were injected with homologous ovary or testis or brain. Those injected with brain failed to conceive for a long period, and when they did most of the offspring were born dead. The females injected with testis or with ovary failed to conceive in more than 300 days. In the females injected with preparations of ovary, the steroid hormones in the homogenate may upset the hormonal balance essential for cyclic activity. Possibly the same explanation may apply to the females injected with testicular tissue, but another interpretation is also possible.

If testicular injections in the male guinea pig result in an immune response, such

injections may sensitize the females. If the immune reaction is anaphylactoid, the organs containing smooth muscle might be affected. If the uterus became sensitized, then, at copulation when a challenging dose of antigen is delivered, the uterus might develop a contracture type of response, which could interfere with implantation. This possibility was explored in the following way. Female guinea pigs were injected with homologous testis or sperm in adjuvant. At varying periods thereafter they were sacrificed, and the uterine horns and the ileum challenged *in vitro* with a dose of guinea pig sperm. About 17 to 21 days after injection, the immunized animal's uterus reacts strongly *in vitro* to the challenge by contracting maximally. In some uteri the contracture was maintained for 4 hours. Research in this direction is continuing.

#### *Studies of Organ Specificity: The Nervous System*

Studies such as those described in the preceding pages have led Mr. Charles R. Wytenbach, a predoctoral Fellow of the National Science Foundation, to ask to what extent immunochemical and radioisotope techniques can be used to investigate what might be termed the "fine" specificities of organs and tissues. For example, will such an approach provide an insight into the factors involved in the development of the distinctive regions of the nervous system? At the outset, Wytenbach is employing immunochemical techniques; few results can be presented, since his investigations have been under way only a few months, during which time he has been concerned largely with methodology. He has reported success in preparing antisera in rabbits against extracts of chicken cerebrum, mesencephalon, and cerebellum; however, the precipitin and absorption reactions, and Ouchterlony serum-agar diffusion tests, performed in preliminary characterizations of the antigen-

antibody systems, indicate identity or close similarity of the antigens in the three antigenic mixtures. Clear-cut regional specificity has not yet been attained. For reasons as yet unknown, preparations of mesencephalon invariably react most strongly with antisera of all types, whereas preparations of cerebellum always give the weakest reaction. These consistent differences cannot be explained on the basis of such obvious possible differences as those in protein content. A recent report by Feldman and Yaffe suggests an obvious possibility for the preparation of more specific antibodies, namely, that organ specificity may be enhanced after the induction of immunological tolerance to antigens of heterologous organs.

#### *A Humoral Factor Regulating Organ Regeneration*

Evidence bearing on the regulation of growth of an organ by its own products has come from several directions. Some of the most intriguing findings have resulted from studies of the regeneration of the liver and, to a lesser extent, the kidney of mammals. Mr. Charles D. Steuart, a medical student at The Johns Hopkins Medical School, recently was awarded a fellowship by the U. S. Public Health Service (Institutional Cancer Teaching Grant) to begin, under Dr. Ebert's guidance, a re-examination of the argument that the growth of the liver or kidney is regulated by the presence in the blood of a tissue-specific inhibitory substance, which according to a recent report by Saetren is thermolabile and nondialyzable. Several investigators have argued, for example, that regeneration may be inhibited by returning to the partially hepatectomized or partially nephrectomized animal an extract of liver or kidney, respectively.

Steuart has begun his study by examining the mitotic activity of the remaining kidney tissue after subtotal nephrectomy. He has confirmed Saetren's conclusion that



under these conditions the rate of mitotic activity increases rapidly, reaching a peak after about 48 hours, and then declines gradually almost to the initial level. Ex-

periments in which macerates of the tissues removed are returned to the operated animals are in progress and will be described in a future report.

## TERATOGENESIS

Often the surgeon is confronted with congenital anomalies and defects which not only pose problems of surgical correction and reconstruction but also raise interesting questions as to their origin. To students of embryology these variants from the normal provide material for study, for such "experiments of nature" have been instructive in elucidating normal developmental relationships. Embryological studies must provide the basis for an analysis of the problems in human teratology. The Carnegie Collection includes a large amount of abnormal material; in this and in other laboratories, the types of embryonic and fetal abnormalities have been described, and the periods of gestation at which they occur have been documented. Further descriptive studies will not discover the causes of human terata. Increasing evidence indicates that no unique principles apply to man; therefore, generalizations concerning mechanisms must be arrived at through experimental studies on other forms. To a considerable extent, teratology is still at the stage where it is concerned with visible end results rather than with the physiological processes that preceded them. Real progress cannot be made until this next big step is taken.

Throughout the year the Collection has been employed by several visiting investigators, who, working independently, but in consultation with the senior members of the staff, especially Dr. George W. Bartelmez, have examined critically four problems in teratogenesis. From the standpoint of their contributions to descriptive embryology and medicine and surgery, the investigations are important, but from the standpoint of analytical embryology, they have significance only as far as they may

form the basis for the design of experiments in the future.

Neither embryologists nor students of medicine have hesitated to use a large technical term when a simple word would suffice; when an investigator is engaged in the study of congenital anomalies this tendency seems to be compounded. The reader will understand that in preparing this brief account the writer has tried to translate the principal findings into non-technical language, with the attendant risk of oversimplification. Specialists interested in the details of the work outlined are referred to the individual investigators or to their publications.

### *Bronchiogenic Cysts and the Theory of Intralobular Sequestration*

In studying the sectioned human embryos in the Collection in the spring of 1957, Professor Edward A. Boyden, of the University of Washington, made a significant contribution to a subject now claiming the attention of those interested in chest diseases. Solitary and multiple cysts of the lungs, filled with air or watery fluid and lined with bronchial epithelium, have long been recognized as congenital malformations, because they have been found in late fetuses and newborn infants. Yet they have never been identified in embryos or in early fetal stages. The problem is complicated by the frequent presence of infection in the lower lobes and by the coincidental occurrence of a systemic pulmonary artery. For these reasons debate has continued, first, as to whether such cysts represent early developmental anomalies or must be attributed to environmental changes of the later fetal and postnatal periods, and, second, as to whether there is a cause-effect relationship between the occurrence of an

accessory pulmonary artery and a bronchiogenic cyst. As Professor Boyden points out, an important goal in the attempt to reach an understanding of these lesions is to "catch" such anomalies in the process of formation. Thus, it is of great interest that he has described cysts in the upper lobes of a 31-mm human embryo and an accessory pulmonary artery in a slightly older 41-mm fetus. Dr. Boyden has described his findings fully, together with a critical evaluation of the theoretical side of the problem, in an illustrated paper submitted for publication in the *Journal of Thoracic Surgery*.

#### *Congenital Malformations of the Heart Associated with Splenic Agenesis*

Enid F. Gilbert, Kinsuke Nishimura, and Bernice G. Wedum, of the Department of Pathology, The Children's Hospital, Washington, D. C., have completed a long-term study of the congenital absence of the spleen in the human, a defect almost invariably associated with severe cardiac malformations. Dr. Wedum has been responsible for the embryological aspects of the study carried on in this Department. After studying monographs, models, serial sections, and serial bromides, Dr. Wedum and her associates have advanced a tentative explanation of the syndrome, which she has described in a manuscript recently completed for publication. The most probable explanation of this combination of lesions appears to be a factor with a wide range of lethality operating at an ovulatory age of about 24 days (horizon xi; 2.5 to 3.0 mm). In its mildest form, only the development of the spleen is suppressed. In the most severe cases, the development of the entire germinal bed of the mesoblastic surface of the coelomic cavity is suppressed, resulting in the absence of the spleen and omental bursa and in the arrest in the development of the primitive tubular heart and the dorsal and ventral mesenteries. It is considered significant that the structures derived from the gut show no

arrest in their development. The timing of these events at 24 to 28 days leads Dr. Wedum to raise the question whether an abortive or faulty ovulation might in some manner influence the developing embryo.

#### *The Anatomy of Clubfoot*

Although clubfoot deformities are a common clinical problem, the results of our present therapy often are far from perfect, possibly because the anatomy of this deformity is unknown or at best controversial. Fourteen specimens have been reported in the literature, most of them more than fifty years ago. The quality of the dissections varied widely, and emphasis was placed almost entirely on the cartilaginous structures. For practical purposes, knowledge of these has been lost to modern orthopedic surgeons, and the clarification of the anatomy of clubfoot is long overdue.

The Carnegie Collection contains eleven clubfoot specimens (*talipes equinovarus*); they almost equal the total number previously reported, and no preceding investigator has seen more than three or four dissected specimens. Dr. George W. Settle, of The Johns Hopkins Department of Orthopedic Surgery and Baltimore Children's Hospital, plans careful gross dissections of them all. At present only four have been completely dissected, along with normal specimens for comparison. X-rays and photographs have been taken, representative specimens of muscle and nerve being removed for histological study. Mr. Richard D. Grill, photographer, has rendered valuable aid in preparing photographic records. Investigation of the possibility of staining these differentially and embedding them in plastic blocks for permanent preservation is under way. In the four specimens dissected the anatomical deformity has been consistent: it is complex, involving seven bones and their articulations, and their muscles and tendons. The keystone deformity, however, is in the shape of the talus. When investigation



of the anatomy is complete, Dr. Settle anticipates that direct surgical correction will be indicated on cases of clubfoot that do respond to conservative-conventional therapy. The problems thereby introduced in the surgery of growing bones and their cartilaginous models will probably have to be investigated experimentally.

*The Embryology of the Nasofrontal Area and Related Congenital Anomalies*

In a manuscript prepared for The Foundation of the American Society of Plastic and Reconstructive Surgery, Inc., Dr. John D. Des Prez, of The Johns Hopkins Department of Plastic Surgery, has reviewed the normal development of the nose and associated structures in order to interpret the findings of a group of anomalies of the nasofrontal area. Thirty normal embryos from the Carnegie Collection were studied. Both the external and internal architecture of the nose was traced through the course of development from the inception of the olfactory placode in the 28- to 30-day-old embryo to the completion of the nasal passage by fusion of the palatal

processes in the 55- to 60-day-old embryo. The stages of development were illustrated by photographs prepared by Mr. Grill.

Eighteen case illustrations have been studied which demonstrate the pattern of segmental development of the nose. Six of them were from the Carnegie Collection, and twelve were clinical cases. Three specimens of total agenesis of the nose are compatible with the interpretation of failure of development of the olfactory placode. The palatal processes were present in all three, and an intact palate was found in the two mature examples. The third was a 7-week old embryo in which the palatal processes were present but not yet fused. This anomaly also may be considered to be the simplest form of choanal atresia (obliteration of the nasal openings into the oral cavity). The usual clinical picture of choanal atresia may be interpreted as an overgrowth of the palatal process on the superior posterior ridge.

Dr. Des Prez's extensive findings, which deal with a number of related congenital defects, could not be summarized further without resorting to technical description unsuited to these pages.

## PHYSIOLOGY OF THE OVIDUCT AND UTERUS

### *Oviduct*

In Year Book 55, Dr. David W. Bishop's investigations of the metabolic conditions within the oviduct of the rabbit were summarized. Since the findings and implications of the research, demonstrating that the tubal fluid of the rabbit is an active secretion, under hormonal control, and that the metabolism of both egg and sperm in the female reproductive tract is probably aerobic, were stated at that time, there is no need to comment further on the series of publications which have appeared during the past year. Interested persons may consult these papers, cited in the Bibliography, for clarification and extension.

### *Uterus*

*The phases of the menstrual cycle and their interpretation in terms of the preg-*

*nant cycle.* In a paper submitted to the *American Journal of Obstetrics and Gynecology*, Dr. George W. Bartelmez has analyzed the phases of the menstrual cycle. He offers evidence that the current terms *proliferative* and *secretory* (for *follicular* and *progravid*, respectively) are misleading inasmuch as they imply certain unsubstantiated deductions and suggest differences from other mammals, differences that do not exist. The supposed resemblance between the mucosa that survives menstruation and the basal zone of other phases vanishes when the reticular framework is stained specifically. In addition to the basal zone, two zones can be recognized during menstruation and repair. The reduction in thickness after the initial menstrual sloughing is due primarily to loss of ground substance from the stroma.

The involution is greatest superficially.

Mitoses are practically absent from the stroma during repair. They are uncommon at first in the epithelia and entirely absent in the basalis. Mitoses in the stroma are not abundant enough to account for the observed increases in the thickness of the endometrium. The outstanding feature of the repair and follicular phases is the increase in stromal ground substance. The glands are not stretched but keep pace with this thickening by cell division. Secretory activity in the preovulatory period has often been demonstrated both histologically and chemically. The myometrium is structurally a muscularis mucosae; its characteristic activity in this phase keeps the highly fluid secretion from accumulating in the glands. The secretion in the postovulatory period is prominent on account of its greater density and because it accumulates in the glands as a result of the change in myometrial activity under progesterone dominance.

The term "follicular phase," widely used in comparative studies, has no misleading implications and emphasizes the controlling ovarian activity. Since secretion is not confined to one phase of the cycle, the terms *progravid* or *progestational* are to be preferred, for they recognize all the adaptive features of an endometrium controlled by the corpus luteum. The thickness of the endometrium and the myometrium of rhesus can be compared in appropriately prepared material. When variations in the size of the several uteri are compensated for, the graph of cyclic changes exhibits two regressions which are not represented in diagrams based on general impressions. There is a slight regression immediately after ovulation and a prominent one before menstrual extravasation.

*Deciduoma formation.* Dr. Vincent J. De Feo's primary aims are to design and carry out experiments that may yield information concerning (1) the hormonal interplay which enables the uterus to acquire and to lose its sensitivity; and (2) the mechanism by which the blastocyst initiates the formation of the decidua. He

believes that studies involving deciduoma formation are useful approaches to these problems.

During the year, attempts were made to produce a "massive" deciduoma, extending from the tubal to the cervical end of the rat uterus, by chemical and physical means. Physiological solutions were perfused into the tubal end of the uterine lumen in varying volumes (1 to 10 ml), hydrostatic pressures (5 to 400 mm Hg), and temperatures (2° to 50° C). Although deciduoma responses were obtained in some cases, no definite correlations could be made. Nor did changes in pH of the perfusate, ranging from 3 to 11, give clear information, though more successes were noted using solutions at the higher pH values.

The intraluminal injection of a small volume (0.1 ml) of saline containing a test chemical was also explored for the possibility of triggering the deciduoma response. Histamine dihydrochloride (10 to 1000 micrograms) was not satisfactory. A 3 per cent solution of pure trypsin, however, was capable of initiating a "massive" response. The uterus became very turgid within seconds after introduction of the enzyme, and the stroma was found (histologically) to be extremely edematous for at least the first 12 hours after treatment. By 24 hours post-treatment, the primary decidual reaction was in progress.

Studies involving uterine mast cells were also undertaken in another attempt to evaluate the role of histamine. These cells are known to release histamine and serotonin, which are believed to contribute to edema formation. In the rat uterus the mast cells predominated in the region of the mesometrial triangle; they were relatively rare in the mucosa. During the estrus, or the pseudopregnant cycle, these cells were quite constant in number. According to De Feo, the intraluminal administration of 10 to 100 micrograms of the drug 48/80, widely used to cause depletion of the mast cell granules and release of histamine and serotonin, initiated



a massive deciduoma response correlated with a loss of uterine mast cells within 24 hours. The drug did not produce the response when given intraperitoneally.

The release of mast cell substances, however, is probably not the trigger for deciduoma formation, as was established by daily intraperitoneal treatment with 48/80 from the first through the fourth days of pseudopregnancy or pregnancy. Although the mast cells were found to be depleted by this treatment, the pseudopregnant uterus nevertheless responded maximally to needle traumatization, and, in the pregnant animals, laparotomy a few days before delivery revealed normal fetuses, no resorptions, and excellent agreement between the number of fetuses and corpora lutea. If the assumption is correct that the deciduoma produced by intraluminal 48/80 is initiated via mast cell products, one may speculate that perhaps substances similar

to those contained in the mast cells may also be released from the mucosa during conditions resulting in decidual cell formation. The failure of the intraperitoneally administered drug may be due to its dilution in circulation, so that the concentration reaching the mesometrial triangle is ineffective in influencing the mast cells to the proper degree before the uterus loses its sensitivity. Timing may be important, for the duration of uterine sensitivity during pseudopregnancy is shorter than is generally realized.

Recent studies using intraluminal trypsin to elicit the decidual response showed that an all-or-none type of relation regarding sensitivity may exist, for treatment on the fourth day of pseudopregnancy gave maximal deciduoma formation, whereas introduction of the enzyme on the third or fifth days gave no gross or histological evidence of a response.

## PHYSIOLOGY OF THE PLACENTA

### *Circulation in the Maternal Placenta*

*Vascular connections between endometrium and intervillous space.* In 1935 Spanner reported that he had counted 94 spiral arterioles supplying maternal blood to a 7½-month human placenta via 488 separate openings into the intervillous space, each arteriole having from 1 to 19 openings into the space by independent terminal mouthpieces. This count was made on a single specimen injected with plastic and subsequently digested to remove the tissues. Twenty years later, Dixon Boyd computed arterial openings in six human specimens. Three from the fourth month had 120, 102, and 156 openings, respectively, and three at term had 320, 310, and 180 openings. The computations were based on extending counts of limited regions of known area to the whole placenta. He recognized two sources of inaccuracy in these counts: first, the openings are more numerous than arterial stems, because of the existence of the multiple terminal mouthpieces; second, the frequent group-

ing of vessels can render any given sample region atypical of the whole. There is no record of any count or estimate of the number of connections in the monkey placenta.

It appeared to Dr. Elizabeth M. Ramsey that a group of pregnant rhesus monkey uteri prepared in recent years in the Department by intravital injection techniques were uniquely adapted for actual counts of vascular connections. Complete serial sections through one or both placentas *in situ* eliminate the sources of error in Spanner's and Boyd's techniques, i.e. fixed tissues which serve to identify landmarks and to differentiate arteries and veins are not destroyed as in Spanner's corrosion preparations; and every vessel is actually seen and counted and its full course from placenta to myometrium traced, obviating extrapolation from a sample that may not be representative, as Boyd himself pointed out. Dr. Ramsey had observed previously that the maternal arteries of the rhesus monkey do not communicate with the intervillous space by multiple mouths, as

in the human; indeed, the reverse not infrequently occurs, and the terminal portions of two adjacent arteries coalesce to enter the intervillous space by a common mouthpiece or "terminal sac."

The facts emerging from the study are: (1) The total number of vascular connections is much smaller than would be anticipated by calculation of relative sizes of human and monkey placentas, even when both primary and secondary monkey placentas are counted. (2) The ratio of communicating maternal arteries and veins is roughly in the order of 1:2, which corresponds to that prevailing in other body tissues. (3) The distribution of both arterial and venous openings is haphazard, with a slightly higher concentration in the central area than at the periphery. (4) A new safety factor to prevent "short cutting" of afferent blood directly to exit channels is recognized in the fairly wide distance between the vascular orifices. This historically troublesome problem of "short cutting" prior to effective circulation through the intervillous space might never have arisen had such plotting of the spread of orifices been done before. (5) The material provides additional evidence of the manner in which afferent blood spurts in a discrete jet from the basal orifice toward the subchorial lake. Dr. Ramsey's preceding reports of observation of this phenomenon cited material in which the injection was effected by artificial pressure. The present injections were accomplished entirely by the maternal blood pressure. These discrete jets under a high head of maternal pressure have been mentioned as a factor preventing short cutting. This factor is still regarded as of prime importance in conjunction with the spread of orifices noted above. (6) It is again observed that maternal arterioles apparently function independently of one another and that not all are delivering blood to the intervillous space at any given moment. The controlling factor determining functional activity is being investigated further. (7) Finally, the role of the marginal drain-

age, asserted by Spanner to be preponderant in the human placenta, is once more shown to be of minor importance in the rhesus monkey.

The above findings have been recorded in photographs and drawings as well as in diagrams and once (C750, primary placenta) in a plastic-sheet reconstruction, prepared by Mr. John M. Goffinet. All previous models of placental vasculature, prepared in the course of the present broad study of circulation in the maternal placenta of primates, have dealt with small, representative areas of interest. Mr. Goffinet's model embraces one entire placenta and the portion of the uterine wall to which it is attached, depicting the total uteroplacental vascular bed of that placenta. This handsome and highly instructive work aroused much interest when exhibited at the meeting of the American Association of Anatomists in Baltimore (1957) and evoked many requests for a description of the technique of production. Numerous models of this type have been prepared in the Department and by workers elsewhere, many of whom came to Baltimore to learn the technique from Mr. O. O. Heard, who perfected it (note in particular the models prepared by Miss Eleanor C. Adams in Boston for the publication of the Hertig-Rock embryos). No description of the methods and materials used, however, is available in the literature. To fill an obvious need, Mr. Goffinet has prepared a description which embodies the cumulative experience of some 20 years' use of the technique by others, in addition to his own conclusions following experimentation with the most recent types of ink and paint and plastic sheets. This useful summary will be found in the section "Apparatus and Techniques."

*Pressure gradients controlling placental circulation.* Morphological studies during the past several years have led to the conclusion, stated in Year Book 55 and elsewhere, that circulation in the maternal placenta of primates is effected by the *vis a tergo* of the maternal blood pressure.



This conclusion is based on the proposition that there is a sharp fall in blood pressure between the uterine arteries and the intervillous space and an additional fall between the intervillous space and the uterine veins. It is further assumed that myometrial contractions throughout pregnancy (Braxton Hicks contractions) enhance this differential by intermittently compressing the uterine veins, thus producing a temporary rise in intervillous space pressure with abruptly increased intervillous space drainage following relaxation of the myometrium. This hypothesis contradicts the traditional belief that the myometrial contractions "squeeze the placenta like a sponge," expressing its content of blood.

The studies of intrauterine pressure carried out by Alvarez and Caldeyro of Montevideo (1950-1957) on human patients at Caesarean section confirm the above hypothesis and supply actual values for some of the components of the system. These workers employed microballoons, inserted into uterine vessels and various portions of the myometrium, and fluid-filled polyethylene catheters, introduced into the amniotic sac and in a few instances into the intervillous space. Pressure values were registered by strain gauges and mechanically recorded. Tokodynamometric studies of myometrial contractions amplified the above data.

The limitations necessarily imposed by clinical material prevent these valuable pioneer investigations from answering many basic questions. This point needs no further elaboration, and it is equally apparent that the Carnegie monkey colony provides an ideal material for establishing the physiological data required for confirmation or, if necessary, modification of the circulatory hypothesis deduced from morphological studies.

By excellent good fortune a two-channel strain gauge and Sanborn recorder were found, in the summer of 1956, to be available at The Johns Hopkins Hospital, Department of Obstetrics, in the experienced

and capable hands of Dr. George W. Corner, Jr., who had been conducting preliminary studies of human intrauterine pressures. Dr. Corner and his colleague, Dr. W. Newton Long, Jr., were willing to collaborate with Dr. Ramsey in the use of the Carnegie monkeys for study of the circulatory problems of mutual interest, devoting their surgical skill to the performance of the operative procedures, and their knowledge of electronics, and that of their technical assistant, Mr. Herbert Stran, to performance of the pressure recordings.

At the end of the first year of this collaboration, certain positive results are already in hand. Since the project has been planned to cover approximately three years, the investigators have "made haste slowly" and to date have devoted primary effort to working out techniques, all of which are entirely new and have required improvisation and progressive modification, and to laying the basic physiological foundations for later work.

Thus, in the fall of 1956, before monkey pregnancies were established after the usual anovulatory summer season, multiple determinations of systemic blood pressure were made in 10 animals. This fundamental piece of physiological information has, oddly enough, never previously been available for the monkey on a satisfactorily wide and systematic basis. Pneumatic cuff determinations being technically impossible in the monkey, the method of direct canulation of the femoral artery was employed, surgically opening the femoral triangle under nembutal or serpasil plus xylocaine anesthetics. The establishment of a mean arterial blood pressure slightly higher than normal human levels was perhaps less important for the project in hand than, first, the determination that blood pressure is essentially the same regardless of whether general anesthesia (nembutal) is employed or tranquilization plus local anesthetic (serpasil and topical xylocaine); and, second, the establishment of systemic arterial blood pressure values for individual females whose intrauterine

pressures were subsequently studied during pregnancy.

These latter studies, carried out in the spring of 1957, have established a standard and comparable method for introducing a polyethylene catheter into the amniotic cavity, the uterus having been exposed by laparotomy. In some half dozen cases a catheter has also been inserted into the intervillous space and/or a uterine vein. Nine animals were used with observations distributed throughout pregnancy.

Although the experiments so far have been performed primarily to perfect techniques, positive results have indicated (1) unequivocal reflection of myometrial contractions in heightened amniotic and intervillous space pressures, (2) correspondence in the variations of absolute pressures in the amniotic and intervillous spaces, (3) inherent amplitude-tonus patterns characteristic on the one hand of individual uteri and on the other of pre-versus post-conversion states in different subjects.

Study has been devoted to methods of caring for monkey "patients" by administration of antibiotics to control possible post-operative infections and of hormones to prevent abortion following the rather drastic operative maneuvers. It has been possible to make repeated observations upon a single monkey by virtue of the efficacy of the post-operative treatment devised. One animal, C753, delivered a normal fetus at term after 4 laparotomies.

Values obtained in the determinations of amniotic, intervillous space, and uterine venous pressures at various stages of pregnancy fall, in general, in line with the observations of Caldeyro. Specific figures are not cited pending observation of sufficient cases to justify generalization. One uniform observation should, however, be recorded, namely that stable curves of characteristic amplitude and interval have not been obtained under 1 to 1½ hours after introduction of the amniotic catheter. Dependable values await the termination of a stabilization period at least this long,

and it has been found profitable to observe the recording for at least 3 hours. The variations in the early part of this time span lead the group to scrutinize with some skepticism reports of other workers based on shorter periods.

*Studies on the Passage of Phosphate  
between Mother and Fetus in  
the Guinea Pig*

In December 1951, Dr. Fritz Fuchs and Dr. Anna-Riitta Fuchs, of Copenhagen, Denmark, completed a year's work in the Department in association with Dr. Louis B. Flexner. Their visit was facilitated by grants from the American-Scandinavian Foundation and from the Carnegie Institution of Washington. At the time their projected research was outlined briefly in Year Book 50, Director Corner looked forward to summarizing the findings when published. During the past year, Dr. Fritz Fuchs presented to the Department a copy of his recently published monograph, a comprehensive account of his extensive investigations of the placental passage of phosphate in the guinea pig, published jointly from the Department of Embryology and the Department of Medical Physiology at the University of Copenhagen. Preliminary studies on the inorganic phosphate in the plasma and the more important results of the experiments dealing with placental transfer have been published by Dr. and Mrs. Fuchs in a series of six papers in the *Acta Physiologica Scandinavica*. These papers are cited in the Bibliography for the report year; three of them more properly belong in Year Book 54, but they are included at this time so that the record may be complete. Dr. Fuchs's monograph is so extensive and so full of descriptive detail as almost to baffle summarization; the writer can do no more than sketch its contents.

With the use of radioactive phosphorus, the passage of phosphate was studied quantitatively both in the direction from mother to fetus and in the direction from fetus to mother, and the transferred amounts were



compared with the quantities of phosphorus retained in fetal growth. In addition, the role of the yolk sac in the transfer has been studied, as have the phosphorus compounds in the placenta *per se*. The detailed accounting of these experiments begins in Dr. Fuchs's sixth chapter, which follows a survey of the pertinent literature dealing with the structure and function of the placenta and the placental permeability to minerals including phosphorus. From the data presented on the concentrations of inorganic phosphate in the fetal and maternal plasma and the transfer from mother to fetus, Fuchs concludes that the inorganic phosphate of the maternal plasma is the main source of phosphate for the fetus. The route by which the transfer occurs is not explained fully, but Dr. Fuchs concludes that the yolk sac does not play any major role. Labeled phosphate, when injected into the umbilical vein of a fetus with intact placental circulation, is partly transferred to the

mother. It amounts, in fact, to about 25 per cent of the quantity transferred in the opposite direction. Fuchs calculates that the amount transferred from mother to fetus is about equal to the amount retained in fetal growth plus the amount returned to the mother. Extensive data are presented on the content of phosphorus compounds in the placenta, and the rate of transfer across the placenta. He computes that about 14 per cent of the plasma phosphate passing through the maternal vessels is removed by the placental cells. The results indicate that the transfer from mother to fetus must require an active transport mechanism, whereas the return of phosphate in the opposite direction may occur by diffusion. A carrier system may be responsible for the transfer from the maternal plasma across the membranes of the chorionic cells, building up there a pool of inorganic phosphate for the fetus. The nature of the carrier system is not known.

### CO-OPERATION IN RESEARCH

As in the past, during the year covered by this report the members of the Department have enjoyed working in co-operation with investigators at other institutions. The undertakings vary widely in scope: in some a true collaborative investigation is being pursued; in others, the Department contributes principally by furnishing experimental facilities and technical assistance. Several joint undertakings have been described earlier, viz. the programs of E. M. Ramsey, G. W. Corner, Jr., and W. N. Long, Jr., and of R. F. Ruth and John I. White, and B. L. Strehler and J. D. Ebert, respectively.

#### *Ionic Environment of the Embryo*

Continuing an interest in the ionic environment of germ cells and early embryonic tissues, Dr. Evelyn Howard, of the Department of Physiology in The Johns Hopkins School of Medicine, has collaborated with Dr. Vincent J. De Feo in an investigation of the ionic content of

the fluid that accumulates in the rat uterus in the proestrus. Their studies have revealed that this fluid, which is presumably of physiological significance as a medium for ascending sperm, has a much higher potassium content than blood plasma, in fact, a potassium content of the order of that of the white of hens' eggs. An effort to determine to what extent this type of uterine fluid is a general mammalian character led to analyses of the uterine fluid found in the pig during the follicular phase, using slaughterhouse material. This fluid showed a similar high potassium but with a wider scatter, presumably due to post-mortem ionic shifts. Attempts to obtain sufficient fluid for analyses from rabbits, mice, and guinea pigs have hitherto been unsuccessful, but are still in progress.

Investigations by Dr. Evelyn Howard with Dr. Bent G. Böving on the ionic composition of the fluid from the early rabbit blastocyst are still incomplete, but tend to confirm in some respects studies

of Lutwak-Mann, and suggest some significant divergence from the composition of maternal blood plasma. Although special qualities of the ionic environment probably have no specific bearing on the problems of differentiation, they are considered of interest inasmuch as they indicate physiological properties of germ cells and embryonic tissue.

#### *Acetylcholine as a Regulator of Cardiac Contractility*

In approaching the mechanism of origin of spontaneous contractility during cardiogenesis, Dr. Robert DeHaan has based his analysis on the hypothesis, formulated by J. H. Burn, that acetylcholine, synthesized within the pacemaker cells of the atrium, is intimately associated with the "firing" of atrial contraction. This concept has interested Dr. Seymour Katsh for several years. During the current year, a paper by Dr. Katsh appeared in the *American Journal of Physiology*, describing the inhibition of isolated auricles of the adult guinea pig by a group of anticholinesterases, substances that inhibit the action of the enzyme acetylcholinesterase, which in turn is known to regulate the accumulation of acetylcholine. Katsh had established previously the order of potency of a group of antiacetylcholinesterases in inhibiting the activity of the enzyme *in vitro*, employing a colorimetric method devised by him. He reports that the same order of potency does not apply in the physiological system, and suggests that the disparity results from differences in penetrability of the inhibitors. He argues further (but does not prove) that the substances exert their effects by permitting accumulation of inhibitory quantities of acetylcholine in the auricles. In continuing the investigation in collaboration with Dr. Jean M. Marshall, of The Johns Hopkins Department of Physiology, Dr. Katsh has examined the effects of the anticholinesterases on transmembrane potentials and mechanical activity of isolated rabbit auricles. Mecholyl ( $10^{-5}$  to  $10^{-4}$  M) produces

a slight increase in the height of membrane and action potentials, but causes a decrease in duration of action potentials, rate, and tension. Carcholin ( $10^{-5}$  to  $10^{-4}$  M) decreases the duration of action potentials, rate, and tension, and at higher concentrations arrests all activity. It should be noted that inhibition and arrest produced by these drugs can be overcome by atropine and epinephrine. The effects of other agents have been studied but, like the ones cited here as examples of the approach employed, at this stage in the study are of primary concern only to the specialist. Details of the research can be found in a paper to be published in the *American Journal of Physiology*.

#### *A Substitute for the Oviduct*

Dr. Böving has also collaborated with a group of investigators in The Johns Hopkins Departments of Obstetrics and Gynecology, Drs. M. L. Carey, L. C. Cian, H. L. Davis, and H. W. Jones, Jr. The primary objective of the research is a practical one; yet it has important basic implications particularly pertinent to Böving's principal investigations. Sterility is often the end result of dysfunction of the oviduct, which in turn may result from a variety of causes. Thus the egg when it leaves the ovary may not be picked up owing to an occlusion of the fimbriated end of the Fallopian tube (or oviduct), or, once in the tube, it may not reach the uterus owing to obstruction or to interference with the motive forces essential to its transport. Although a number of possible solutions to this problem have been proposed and several operative procedures tested extensively, including the use of polyethylene tubes, the over-all chances for a successful term pregnancy are approximately 1 for every  $6\frac{1}{2}$  operations. What is required is the construction of an organ to substitute for the malfunctioning tube. It must (1) entrap the ovum; (2) provide a patent canal to the uterus; and (3) furnish a motive force to transport the egg.



Inert materials generally fail because they do not fulfill the third requirement.

One organ appears to promise fulfillment of these conditions, a part of the intestinal tract, the terminal portion of the ileum. It is expendable, it lies in proximity to the reproductive organs, and, despite certain differences which might prove troublesome, it is evidently similar to the oviduct in many ways. Although the first test of this idea, which was made in rabbits, failed, the over-all results of the experiment are heartening. A 4- to 5-cm-long segment of ileum substituted for the oviduct showed the following favorable features: (1) the egg was deposited in the segment (at operation, the ovary was included in the lumen); (2) the lumen remained patent throughout its extent; (3) the segment maintained a favorable alkaline environment; (4) peristaltic waves traveled from ovary to uterus; (5) after 10 months' exposure to the ileal segment, the endometrium was not damaged; and (6) sperm were viable in the segment for at least 20 hours. In view of these favorable circumstances, why were no gestations obtained? Böving and his co-workers have advanced a likely explanation. As was discussed earlier, in the rabbit the oviduct has the essential function of producing one of the egg coverings, the *mucolemma*, a covering required for successful implantation. Several lines of evidence suggest that this tubal function is not essential in the primates, including man; therefore, at this writing tests are being made in the monkey.

#### *Experimental Endometriosis*

The Department continues to participate in the long-term experimental study of endometriosis being conducted by Professor R. W. TeLinde and Dr. Lawrence R. Wharton, Jr., of The Johns Hopkins Medical Institutions, and Dr. Roger B. Scott, of Western Reserve School of Medicine, by furnishing facilities and technical assistance. Once again, this study has im-

portant basic and practical aspects. Specifically it is concerned with external endometriosis, the condition in which fragments or foci of endometrial tissue (the membrane that lines the cavity of the uterus) occur in other places in the body, like the external surface of the uterus, the ovary or bladder, or intestine. Among the unusually interesting features of external endometriosis in the human are the following: as a benign condition it is limited in its clinical manifestations to the years of menstrual function; it has not been identified prior to the onset of menstruation—and becomes atrophic at menopause. Delayed childbearing predisposes to its prevalence, whereas pregnancy has a salutary effect, the disease becoming essentially quiescent. These and other observations, including the evidence presented earlier by this group that fragments of endometrium discharged at menstruation in the monkey are viable, have led to the current study of the effects of the ovarian hormones on experimental endometriosis.

In this investigation the islands of ectopic endometrium are considered as "miniature uteri," ordinarily subject to the same influence from circulatory hormones as the uterine endometrium. Fibrotic encasement of the "islands" might block hormonal uptake and response, leading to stagnation of the tissue. Endometriosis which is fully responsive to hormones could bleed and separate when uterine bleeding occurs, allowing for local growth and conceivably local and distant spread via the lymphatics. In the monkey, endometrium was transplanted to four areas in the body cavity, and the transplants were studied after the administration of various combinations of ovarian hormones. Since the details of the study will be reported shortly in the *American Journal of Obstetrics and Gynecology*, only the major findings will be cited here. In all the animals receiving hormones, there was a rough correlation between the normal bleeding of the uterus and bleeding in the transplants. Local growth of ectopic endometrium occurred

chiefly in the group of animals that were treated with a constant dosage of estrogen and given progesterone intermittently. Growth was local and not disseminated; therefore, it is postulated that disseminated growth may be the result of repeated retrograde menstruation from the parent endometrium.

Other research in progress by Drs. Wharton and TeLinde includes an examination of the argument, based on studies by others in the rabbit, that removal of the uterus produces failure of ovarian function. Since indirect evidence to the contrary exists for the human, experiments must be conducted in primates. The work is not complete, but it appears that in the monkey after hysterectomy the ovary will function as before provided that an adequate blood supply remains.

### *Additions to the Collection of Human Embryos*

During the year, Dr. Elizabeth M. Ramsey examined 105 specimens sent by 25 institutions and physicians from 10 states, the District of Columbia, and Alaska. Of the 105 specimens, 79 were discarded as of no research value, at the end of 3 months after reporting to the donor and in the absence of instructions to the contrary. Two specimens were returned to the donors, and 24 specimens were preserved for future research.

The Director is happy to record the receipt of a well planned album of photographs of an early human embryo, deposited in the Collection by Professor Ira D. Hogg, of the Department of Anatomy at the University of Mississippi Medical Center.

## APPARATUS AND TECHNIQUES

### *A Nonnutrient Culture Medium for Amphibian Embryonic Tissues*

Holtfreter's solution has long been the standard medium for the culture of tissues explanted from amphibian embryos. Dr. Steinberg has found it difficult, however, to culture in it explants from gastrula stages of several species of amphibians. Niu and Twitty's solution, which has been reported to be superior to Holtfreter's solution, proved no better for Steinberg's purpose. Only a small proportion of tissues explanted from gastrulae of *Triturus pyrrhogaster*, a Japanese newt, have survived in Niu and Twitty's solution. Consequently, Steinberg undertook the development of a more satisfactory culture solution.

Both the culture solutions mentioned above contain bicarbonate. Acidometric titrations of such solutions, repeated daily, have shown that within two or three days after the fresh solution is transferred to a covered culture dish the bicarbonate is converted quantitatively to the hydroxide with the liberation of carbon dioxide. The result is a loss of buffer capacity (in Holt-

freter's solution) and a rise in pH. Furthermore, the utility of the bicarbonate is thrown into question, since it is present only for the first two or three days, at most, of a two- to three-week culture period.

Niu and Twitty's solution contains, in addition to bicarbonate, a phosphate-pair buffer. The presence of phosphates and the divalent cations calcium and magnesium in the same solution necessitates sterilizing these components separately and mixing them only after cooling to avoid precipitation of calcium and magnesium phosphates. Bicarbonate is sterilized as yet a third solution before addition to the other components. The pH of Niu and Twitty's solution before evolution of carbon dioxide from the bicarbonate is approximately 7.8. After carbon dioxide loss, it is approximately 8.1, which is believed to be unfavorable.

In an effort to improve upon the above solutions, Steinberg first determined the pH of the capsular fluid of *T. pyrrhogaster* eggs by injecting appropriate indicator dyes into the capsular fluid. These injections showed that at all stages from the



uncleaved egg to fairly advanced larval stages the fluid normally surrounding the embryo has a  $pH$  of 7.3 to 7.4, which should be the proper  $pH$  for a culture fluid, at least for this species.

The presence of phosphates is believed to be incompatible with that of calcium and magnesium; moreover, under some conditions exogenous phosphates are toxic to animal cells. A substitute for the phosphate buffer was found in Tris (hydroxymethyl) aminomethane (Sigma Chemical Company). This buffer has been found by others to permit the optimal activity of a number of enzymes and has been used successfully in tissue cultures of mammalian cells. Bicarbonate was omitted from the medium, and the  $pH$  was brought to approximately 7.4. The concentrations of the other salts are those used by Niu and Twitty. The final formula is given below. The salts are conveniently kept as stock solutions made up in glass-distilled water to facilitate rapid preparation of the medium. The entire solution is made up in one flask and autoclaved, there being no  $pH$  change in the process. If antibiotics are to be used (optional), a portion of the water is reserved, and just before use the antibiotics are dissolved in it, the solution is sterilized by Seitz filtration, and the sterile solution is added to the major portion of the medium. Since this medium was adopted, not one explant has failed to survive for the entire period of culture.

#### Formula

17.0% NaCl .....	20 ml
0.5% KCl .....	10 ml
0.8% $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ .....	10 ml
2.05% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ .....	10 ml
1.00 N HCl .....	4 ml
Tris .....	560 mg
(Streptomycin sulfate) .....	50 mg
(Penicillin G-sodium) .....	50,000 units
Glass-distilled $\text{H}_2\text{O}$ .....	946 ml

#### *A Simple Method for the Removal of the Vitelline Membrane from Amphibian Eggs*

Townes has reported that solutions of the proteolytic enzyme papain are capable

of digesting the vitelline membrane of amphibian eggs. In repeating his work, Steinberg found that papain without the addition of cysteine hydrochloride, which normally serves as an activator of the enzyme, has no effect upon the vitelline membrane. When a crystal of cysteine hydrochloride was placed near an egg immersed in a papain solution, the vitelline membrane was digested almost instantaneously adjacent to the crystal. This observation suggested a direct effect of the cysteine hydrochloride rather than an enzyme activation. Therefore, the experiment was repeated, omitting the enzyme. Cysteine hydrochloride alone proved to be astonishingly effective in digesting the membrane of several species of both anurans and urodeles. In practice, it is a simple matter to drop a very small crystal of cysteine hydrochloride on or near an egg, and then flush it away rapidly with a stream of culture fluid from a pipette. The demembrated embryos subsequently develop normally. This procedure makes available to the embryologist for manipulative procedures naked and uninjured eggs of any stage beginning with the freshly laid uncleaved egg. The technique should be of considerable advantage for work at pre-gastrula stages, when mechanical removal of the vitelline membrane without injury to the egg is difficult.

#### *An Aid to the Gentle Dissociation and Reaggregation of Tissue Cells*

In certain experiments involving the dissociation of a tissue into its cellular components followed by the reaggregation of these free cells to reconstitute a tissue, it is desirable to minimize mechanical disturbance to the cells. In Steinberg's work in disaggregating cells of amphibian gastrulae, using ethylenediaminetetraacetic acid, disodium (EDTA), or high  $pH$ , he has been able to dissociate tissue fragments and subsequently allow them to reaggregate, without any mechanical disturbance whatsoever, by means of rings machined from stainless steel.

A number of thin-walled steel rings of varying diameter were made. Tissue fragments are dissociated by immersion in solutions of EDTA or of high pH contained in cups of 5-ml capacity (of the type designed for use with a pH meter) and floored with 1 per cent agar. A ring with a lumen slightly greater than the diameter of the pile of dissociated cells is placed over the pile of cells carefully, caution being exercised not to crush any peripheral cells. The solution for effecting dissociation is pipetted out of the dish, a minute volume of the solution remaining in the lumen of the ring to protect the cells beneath from exposure to a meniscus or from any disturbance whatever. Fresh culture solution is then pipetted into the dish, after which the ring can be removed gently. The tiny remaining amount of solution used for disaggregation is now dispersed in the overwhelmingly greater amount of culture solution, and the cells proceed to reaggregate.

This technique may be applicable to experiments in which other tissues are employed, provided that mechanical agitation is not required to cause final dissociation of the cells. Its advantage is that the cells are not exposed to any physical violence during dissociation, and thus may be expected to suffer minimally in the process.

#### *Preparation of Avian Hemoglobin*

As was reported earlier, in order to purify avian hemoglobin, Mr. Robert G. Beard found it necessary to devise a technique for the removal of intact nuclei of avian erythrocytes. The events leading to the formulation of the method and the method itself are described, for it should prove of value not only to persons concerned with the extraction of hemoglobin but also to those interested in preparing isolated nuclei. Beard had found previously that the lysis of washed avian red blood cells with an equal volume of distilled water led to the formation of a thick nucleoprotein gel that was difficult to remove and that caused the loss of

approximately 50 to 60 per cent of the available hemoglobin in a given lysate. Studies involving the mincing of the gel, followed by squeezing through cheesecloth and high-speed centrifugation, failed to yield a suitable recovery of the hemoglobin. Aluminum hydroxide cream was also employed to help bind and sediment the gel particles. Marshall and Welker's discovery that oxyhemoglobin is not bound by aluminum hydroxide cream in contrast to the rather strong binding of other proteins was helpful here. Difficulties in keeping the hemoglobin oxygenated led to some loss, however, for reduced hemoglobin is significantly bound by the cream. Further studies using carbon monoxide hemoglobin instead of oxyhemoglobin should clarify this point. In general, two treatments of a lysate with  $\frac{1}{4}$  volume of aluminum hydroxide cream followed in each case by centrifugation for 1 to 2 hours at 3000 rpm in the refrigerated centrifuge serve to clarify the hemoglobin solution completely. Ultracentrifugal studies have not yet been performed to determine whether all the smaller subcellular particulates are removed by this treatment.

In another effort to remove the nucleoprotein gel, deoxyribonuclease was employed. In these experiments, the enzyme was added to lysates of red blood cells to which had been added solid magnesium chloride to a final concentration of 0.01 *M* with respect to magnesium. The addition of 2 mg of enzyme per liter of lysate or of 5 mg per liter of lysate caused a marked decrease in the viscosity of the solution, but not a complete removal of the gel, even after a period of hydrolysis of 6 days at 2° C. Although this method was a distinct improvement over previous ones, the success was not great enough to warrant its use.

Several attempts were made to remove the nucleoproteins by taking advantage of their solubility in 1 *M* sodium chloride (6 per cent) and their relative insolubility at physiological tonicities (1 per cent). Solid sodium chloride was added to a final con-



centration of 6 per cent. The amount of nucleoprotein present was great enough to necessitate the addition of 3 liters more of 6 per cent sodium chloride to effect its complete solution. This highly viscous solution was then poured, with stirring, into 5 volumes of distilled water to reduce the salt concentration to 1 per cent in order to precipitate the nucleoproteins as sticky threads. The final volume of slightly less than 24 liters contained only about 200 grams of hemoglobin. The problems involved in trying to concentrate large volumes of dilute protein solution led Beard to abandon this method of purification. Nor was the method of the Harvard group utilizing mercury and zinc ions to precipitate proteins from dilute solution satisfactory in this system.

The only practical attack on the problem appeared to be as complete a removal of the intact nuclei as possible in a medium whose salt concentration was maintained at about 0.9 per cent sodium chloride to keep the nucleoproteins from being extracted from the nuclei. Homogenization of washed red blood cells in saline did not yield appreciable results. Since a gentle method was desired, the Waring Blendor was contraindicated. The successful method finally devised is as follows: (1) Wash red blood cells from freshly collected whole (oxalated) blood 5 times with 0.9 per cent sodium chloride to remove plasma proteins. Also siphon off white cells between second and third centrifugation. (2) To 1 volume of sedimented red blood cells, add 1 volume of 0.9 per cent sodium chloride containing 1 gram saponin per liter; also add  $\frac{1}{4}$  volume of toluene. Mix well by stirring, and let stand in the cold overnight, stirring occasionally. (3) Centrifuge in the bucket head of the refrigerated centrifuge for 2 hours at 2000 rpm. Siphon off the top layer of toluene and also the pad of denatured proteins; discard it. Siphon off the next layer (hemoglobin in saline); retain it, and add to the sediment of whole nuclei an equal volume of 0.9

per cent sodium chloride; resuspend, and centrifuge again. The first and second hemoglobin supernatants are pooled and used as the source of hemoglobin to be further purified by adsorption with aluminum hydroxide cream, high-speed centrifugation, and crystallization by the addition of ethanol in the cold. (4) The nuclei may be stored under a small amount of toluene, or the nucleoproteins may be extracted from the nuclei after several further washings with 0.9 per cent sodium chloride to remove the residual hemoglobin clinging to them.

This preparative method removes the nuclei cleanly and completely as far as has been determined. Microscopical examination of fresh and stained preparations shows that the cell walls were broken and that the nuclei remained intact and in optically good condition.

#### *Plastic-Sheet Method of Three-Dimensional Reconstruction*

The modeling technique described below by Mr. John Goffinet has been used in this laboratory for many years. We believe it to be of particular value in studies for which solid models are impractical, e.g. studies of the vascular patterns of the uterus and the placenta.

The basis of the technique consists in drawing or painting enlargements of serial tissue sections on sheets of clear plastic (acetate). The transparent plastic enlargements are then arranged in serial order to give a three-dimensional effect.

An ordinary photographic enlarger is suitable for making most reproductions. An Edinger projector, if available, is excellent. If the model is to be constructed from opaque sections, a camera lucida should be used. The histological slides are placed in the enlarger and projected at the desired magnification, the structures to be modeled being traced from this projection.

The essence of illustrational clarity lies in the elimination of unnecessary detail. For example, in models of vascular sys-

tems we try to minimize all nonvascular structures. Basic accuracy must not be sacrificed, however. For this reason, it is good policy to do the initial enlargements as pencil drawings on tracing paper (with detail *ad libitum*), thus permitting checking against the original sections for accuracy of interpretation. A second tracing is then taken from the paper to the plastic transparencies.

No attempt is made to align the sections when the initial enlargements on tracing paper are taken; it has proved more practical to wait until the plastic transparencies are prepared. The alignment is effected by superposing a plastic sheet on a paper tracing, doing a second tracing on the plastic, and then placing the next succeeding paper tracing on the plastic, so that the outline of the section is lined up with the previous section. The corners of the second plastic transparency are aligned with those of the previous one. In this way a stack of alternating paper and plastic transparencies is built up, the paper tracings being placed according to landmarks on the section outlines, the plastic sheets being placed by alignment of their corners. The papers are, of course, removed after the model is completed.

In order to preserve the accuracy of the third dimension the plastic transparencies must be separated by a scale distance. The distance can be easily calculated by multiplying the thickness of the original tissue sections by the magnification of the model, and then subtracting the thickness of the plastic sheet. For those models in which this distance is found to be greater than

the thickness of the plastic transparency, simple cardboard mats may be used as spreaders. They may be made simply and economically by stapling the corners of four cardboard "sticks" of appropriate length and width.

Monsanto Chemical Company's Plastics Division (Springfield, Massachusetts) makes a very suitable plastic sheeting, called Vuepak. It comes in 20 by 50 inch sheets, 0.015 inch thick. Plastics of different types and thicknesses are available. Paints and inks are rather a problem, but a satisfactory solution can usually be attained with a little experimentation. If line drawings only are desired, ordinary India ink is excellent. If a clear, painted area is desired, Craftint (Craftint Manufacturing Company, Cleveland, Ohio) permanent ink for plastics, diluted with Craftint thinner, will probably prove most satisfactory. This acetate-soluble ink has the disadvantage of warping the plastic if applied to too large an area. If an opaque area is desired, show-card paint, with a few drops of aerosol added, suffices. Water paints will not stick to the plastic ("creeping") unless a wetting agent, such as aerosol, is added.

The plastic transparencies must be protected from dust and from warping. Since they are easily scratched, Goffinet recommends that they be separated from each other by sheets of soft paper when stored. Spreaders or frames should be removed. If spreaders are left in place for long periods of time, the plastic transparencies will warp.

### STAFF ACTIVITIES

The increasing volume of scientific research in the past several decades has been paralleled by increasing pressures for specialization. In both undergraduate and graduate education, there has been an unfortunate tendency to stress, above all, intensity of effort and single-mindedness of purpose in mastering the subject matter of a specialized subject within a scientific

field. But there is a growing recognition among embryologists, at least, that the major conceptual advances of the coming years will depend in large part on their comprehension of progress in several other fields, notably biochemistry and biophysics, genetics and immunochemistry. Each investigator must strive not only to increase knowledge in his own subject and become



aware of current developments in other fields, but he must also reciprocate by making his own findings and ideas clear to others. The diffusion of knowledge, for both general and scientific audiences, is an integral part of a scientific program. It is practically an aphorism that the preparation of a synthetic article or lecture benefits the author as well as his audience, because it enables him to see his own work in proper perspective, revealing gaps in his knowledge and suggesting new experiments.

Readers of these reports will be pleased to learn that the honorary degree Doctor of Laws was conferred upon Dr. George W. Corner by Temple University on November 3, 1956. During the year Dr. Corner gave several addresses, including a lecture entitled "The Place of Anatomy in Medical Education" at the dedication of the University of Wisconsin's Charles R. Bardeen Memorial Laboratories. He presented the Ayerst Lecture of the American Society for the Study of Sterility on the subject "Laboratory and Clinic in the Study of Sterility." His other activities included attendance at the Ciba Foundation Conference on Problems of Aging, held in London in July 1956.

Dr. Robert K. Burns presented lectures on the general subject of transformation of the embryonic gonad in the opossum under the influence of sex hormones at the Conference on Endocrines in Development held at Shelter Island in July 1956, and at a research conference conducted under the auspices of Florida State University in Tallahassee during April 1957. Staff and students at the University of Florida's Department of Anatomy and the Biology Department of The Johns Hopkins University constituted the audience at other lectures.

During the summer of 1956, Dr. David W. Bishop presented a seminar at the Ciba Foundation in London.

Dr. Bent G. Böving delivered two lectures at Jefferson Medical College during the month of February 1957. He spoke

on the topic "Rabbit Blastocyst Spacing and the Hormonal Implications" before the Endocrinology Seminar, and chose as the title of his lecture for the Obstetrics and Gynecology Seminar "The Regulation of Trophoblast Invasion." In addition, he spoke at the annual meeting of the American Association of Anatomists, a meeting in which Dr. Elizabeth M. Ramsey also took part. Dr. Ramsey's contribution took the form of a demonstration of her research, "The Flow of Blood through the Primate Placenta." Several members of the staff attended these meetings, held in Baltimore, as time permitted.

Other members of the group also took part in scientific meetings and described their current research in seminars and public lectures. Drs. De Feo, DeHaan, and Steinberg represented the Department at the Conference on Cellular Biology, Nucleic Acids, and Viruses held by the New York Academy of Sciences early in 1957. Dr. De Feo also attended the annual meeting of the Endocrine Society. Dr. DeHaan spoke before the Maryland Section of the Society for Experimental Biology and Medicine on the subject "The Serological Determination of Developing Muscle Protein in the Regenerating Amphibian Limb." He has been invited to offer a graduate-level seminar, "Problems in Developmental Biology," in the Department of Anatomy, The Johns Hopkins University School of Medicine. Dr. Steinberg lectured on recent developments in his research program at the University of Wisconsin and to a group at The Johns Hopkins University School of Medicine.

Dr. R. F. Ruth represented the Department at two conferences, The New York Academy's Conference on Immunology and Cancer, also attended by Mr. Robert G. Beard, and the Tenth Annual Research Conference of the Biology Division, Oak Ridge National Laboratory. The theme of the latter conference was "Antibodies, Their Production and Mechanism of Action." While at Oak Ridge, he lectured to the staff of the Biology Division, his

subject being "Cytochemical Morphology of Antibody Production."

The Director served as a member of the Executive Committee of the Society for the Study of Development and Growth; in June 1957, he was elected to the Presidency of that Society. During the spring of 1957, he was named to the Editorial Board of the *Journal of Embryology and Experimental Morphology*. He continued to serve during the year as a member of the Committee for Basic Biological Research on Aging, of the American Institute of Biological Sciences; in this capacity he participated in the organization of the Conference on Basic Problems of Biological Aging, serving as Chairman of the session on developmental aspects of aging. The *Proceedings* of this conference will be published. In addition to speaking before several staff and student groups in The Johns Hopkins University and in the Embryology Course at the Marine Biological Laboratory, Woods Hole, Dr. Ebert presented the following lectures: at Princeton University and Goucher College, he discussed "The Acquisition of Biological Specificity." At the Annual Cancer Research Conference of Harvard University held at Endicott House, Dedham, Massachusetts, his subject was "Dissociation and Reassociation at the Molecular Level." The same topic, in a form more suitable for a general audience, was presented at a meeting of the Society for Experimental Biology and Medicine, held at Fort Detrick, Maryland. A general lecture, "The First Heart Beats," was given at the University of Florida. The Director took part in the

Symposium on Embryonic Nutritional Requirements and Utilization, sponsored by the International Institute of Embryology, and the Symposium on Cytodifferentiation sponsored by the International Union of Biological Sciences, both held at Providence, Rhode Island, during July 1956. The papers presented at these symposia have been prepared for publication. Other members of the group who attended several of the meetings at Providence included Dr. Burns, Dr. DeHaan, Dr. Steinberg, and Messrs. Beard, Wilt, and Wyttenbach. Dr. Ebert also served as Chairman of the Work Conference on Immunology and Development held at Bar Harbor, Maine, in August 1956, a conference in which Dr. Katsh was an active participant.

*Seminars.* The Embryology Seminar organized by the Department in collaboration with Professor Willier and his associates at The Johns Hopkins University brought together a gratifying number of scientists from research institutions and universities in the Baltimore area to discuss recent advances in experimental embryology. The roster of speakers included several distinguished visitors: Professor F. W. Rogers Brambell, Dr. A. W. H. Braden, Dr. André Glinos, Dr. Aron Moscona, Professor C. H. Waddington, and Dr. Edgar Zwilling. During the year the staff had the opportunity of hearing special lectures given at The Johns Hopkins University by Professors Honor B. Fell, P. J. Gaillard, P. B. Medawar, and Etienne Wolff, all of whom also visited the Department to exchange views with individual members of the staff.

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- Wilkie, D. R. See Csapo, A.
- Wolfe, H. R. See Ruth, R. F.

## PERSONNEL

Year Ended June 30, 1957

(Including those whose service began or ended during the year)

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George W. Bartelmez, Consultant in Embryology  
 David W. Bishop, General Physiology  
 Bent G. Böving, Physiology  
 Robert K. Burns, Experimental Embryology

Robert L. DeHaan, Experimental Embryology  
 James D. Ebert, Director  
 Elizabeth M. Ramsey, Research Associate and Pathologist  
 Royal F. Ruth, Cytology and Immunology

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Louis B. Flexner, Philadelphia  
 Arthur T. Hertig, Boston  
 Chester H. Heuser, Augusta, Georgia  
 Samuel R. M. Reynolds, Chicago

*Fellows*

Vincent J. De Feo, Fellow of the U. S. Public Health Service  
 Louis E. DeLanney, Fellow of the Carnegie Institution of Washington  
 Seymour Katsh, Fellow of the Population Council  
 Jacques Mulnard, Fellow of the Rockefeller Foundation  
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 Pieter A. deVries, San Francisco  
 Edward Roosen-Runge, Seattle  
 Bernice Wedum, Washington, D. C.  
 Emil Witschi, Iowa City

*Students* (in co-operation with The Johns Hopkins University)

Robert G. Beard (Graduate, Biology)  
 John Goffinet (Medicine)  
 Charles J. A. Schulte, III (Undergraduate, Biology)  
 Charles Steuart (Medicine)  
 Fred H. Wilt, Fellow of the National Science Foundation (Graduate, Biology)

Charles R. Wytttenbach, Fellow of the National Science Foundation (Graduate, Biology)

*Clerical and Technical Staff*

George Boettinger, Porter  
 William E. Bouchat, Assistant Recorder  
 Harriet L. Caspari, Technician<sup>2</sup>  
 William I. Cleary, Recorder  
 Cosette Coffman, Technician<sup>3</sup>  
 Lloyd Crane, Technician  
 Doreen Davis, Illustrator<sup>4</sup>  
 William H. Duncan, Technician  
 Wilbur F. Garde, Assistant Recorder  
 Richard D. Grill, Photographer  
 William Hamilton, Machinist<sup>5</sup>  
 Wilfred D. McCleary, Technician  
 Marianne Jacobs Moore, Technician  
 Anna M. Pasterfield, Caretaker  
 Margaret Proctor, Secretary  
 Arthur G. Rever, Office Manager  
 Doris G. Smith, Secretary  
 John L. Wiser, Machinist

*Special Technical Assistant pro Tempore*  
Joseph P. Drane

<sup>1</sup> For others associated with research activities of the Department, consult section "Co-operation in Research."

<sup>2</sup> Retired June 30, 1957.

<sup>3</sup> Resigned June 15, 1957.

<sup>4</sup> Resigned June 30, 1957.

<sup>5</sup> Resigned February 15, 1957.





D E P A R T M E N T O F G E N E T I C S

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*Cold Spring Harbor, Long Island, New York*

M. DEMEREC, *Director*



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## INTRODUCTION

Our civilization is moving forward at an accelerated pace, with science as the primary force in its rapid progress, and biology contributing an important share. Genetics, in the forefront among the biological sciences, has arrived in recent years at discoveries that throw new light on the basic mechanisms operating in hereditary systems and on the role of these systems in organic evolution. Newly developed methods, too, have opened up important pathways for the future progress of genetical research. Our Department is taking a significant part in these endeavors. The accomplishments of the past year are summarized briefly in this report.

### RESEARCH

The study of controlling elements in maize has been continued by McClintock, with a system of elements that control gene action at two known loci. This system is not related to the *Ds-Ac* system, but the genes affected are ones that, in other cultures, have come under its control. The number of recognizable elements comprising the system, the changes that occur in their modes of expression, and their capacity for transposition have been examined. McClintock has also carried forward her investigation of a fragment chromosome that exhibits strikingly aberrant behavior in both somatic and germinal cells. The fragment initiates many different types of change in chromosome organization in somatic cells, and in meiotic cells it induces an unorthodox type of crossing over.

Kaufmann and Gay have proceeded with their analyses of organizational patterns of the hereditary materials in higher plants and animals, combining cytological and cytochemical methods. Attention has centered round the fine structural patterns revealed by the electron microscope, and the changes effected therein by treatment of fixed cells with nucleases and proteases

having known specificity of action on nucleic acids and proteins. Some of the findings support their earlier assumption that the chromosomes consist of continuous fibrillar elements, apparently proteinaceous in nature, to which the nucleic acids are presumably attached laterally. Effects of treatment of living cells of the plant *Tradescantia* with ribonuclease during meiotic stages emphasize the significance of ribonucleic acid in the maintenance of chromosome form and structure, and also suggest the possible role of this nucleic acid in the processes of crossing over and recombination. A study of spermiogenesis in *Drosophila* has yielded additional information about the part the nucleus plays in the production of cytoplasmic organelles during differentiation of the mature spermatozoon. Observed syncytial associations of related spermatids indicate the existence of pathways for possible intercellular co-ordination and co-operation. The reorganization of nucleic acids and proteins as the spermatid is transformed into the spermatozoon is commanding special attention, in an effort to lay the foundation for a study of the fine structural changes caused by ionizing radiations in the chromosomes of this fly.

McDonald has continued her studies of intracellular deoxyribonucleases, which appear to be correlated with deoxyribonucleic acid synthesis and metabolism, and hence with cell division. Until these enzymes have been isolated and characterized, however, their exact role in life processes cannot be definitely established. Her efforts to purify the deoxyribonucleases of calf spleen, carried on intermittently since 1952, have been greatly hampered by the high content of blood found in this tissue. During the past year she has therefore made a survey of the deoxyribonucleases of various other tissues, in order to locate a better source material. This has been found in salmon testes, and procedures for ex-



traction and purification are now being developed.

Hershey's group has obtained new evidence that the "chromosome" of bacteriophage T2 contains nucleic acid exclusively, and that it can multiply in functional form in bacterial cultures containing chloramphenicol, which inhibits synthesis of all phage proteins.

Streisinger has investigated the nature of reverse mutations in bacteriophage, and has found that mutations resulting in loss of adsorptive capacity in the phage T2 $\lambda$  take place at any of a large number of sites within a gene, whereas reverse mutations, which restore the adsorptive capacity, occur at precisely the sites of the forward mutations from which they derive. He has determined, however, that not all reverse mutants are identical with the original, unmutated form. Three classes of phenotypically different reverse mutants were isolated from one particular mutant, all resulting from reverse mutations at the same site as that of the original forward mutation. The mutations studied by Streisinger control the specificity of attachment elements located on the phage tail. He has obtained evidence that the number of elements per phage tail is small, two being the most probable number. Wassermann, working with Streisinger, has found that the attachment specificity of phages T5 and PB (two related phages which are unrelated to T2) is controlled by a number of independent loci. Phage PB partially excludes T5 in mixed infections. The inheritance of the exclusion property was shown to be fairly complex.

Demerec and his collaborators have continued their studies of genetic mechanisms in the bacterium *Salmonella typhimurium*, employing methods based on the phenomena of transduction and transformation. They have constructed stocks having various combinations of genetic markers representative of a cystine locus and four tryptophan loci which are so closely linked that they are carried together in one trans-

ducing fragment. Preliminary experiments with such stocks have shown that three separate portions of a fragment may be incorporated simultaneously into a newly formed bacterial chromosome. If incorporation is accomplished through a process similar to crossing over in higher organisms, six simultaneous crossovers would be required to produce the combinations of markers observed in these experiments. Ozeki has obtained evidence that a transducing fragment is not a randomly selected section of bacterial chromosome. His findings suggest that the chromosomes of donor bacteria are partitioned during the lytic process into small sections, in some regular way and at predetermined locations, producing for any one region uniform transducing fragments. Attempts have been resumed to bring about transformation in *Salmonella*. An experiment in which *tryD-7* bacteria were combined with DNA extracted from wild-type bacteria produced a large number of variants carrying both the wild-type allele of *tryD-7* and a new allele at the *tryB* locus, which is very close to the *tryD* locus. Thus our findings are similar to those reported two years ago, namely, "transformation" to wild type in the marker used for selection, and coincidental mutation at a locus near the marker locus.

Continuing her research with *Aspergillus* begun at the University of Glasgow, Käfer has investigated the process of haploidization, and has attempted to isolate aneuploids from a well marked diploid strain, in which markers on all chromosomes made it possible to determine the number of disomic chromosomes and to observe the breakdown of the unstable aneuploids into haploids. The results show that most haploids arise as a consequence of stepwise loss of one member of the various chromosome pairs. Of the intermediate aneuploid stages, those with a large number of disomic chromosomes are less viable than those having only one disomic pair, and even the latter are out-

grown when in competition with the derived haploid.

Again this year, the research program of the Department was expanded with the help of three grants. Two of them were received from the U.S. Public Health Service, one in support of research by Kaufmann and the other in support of Hershey's work. A third grant was received from the American Cancer Society.

#### STAFF

This year has brought about no changes in the major scientific staff of our Department, although there have been a considerable number among the research assistants, who usually stay with us for a year or two before taking up graduate studies.

The Department had the following fellows in 1956-1957: Drs. Joseph D. Mandell, Etta Käfer, and Atif Sengün, who held Carnegie Institution Fellowships; Dr. Royston C. Clowes, Fellow of the Damon Runyon Memorial Fund; and Dr. Andrej W. Kozinski, Fellow of the Polish Academy of Sciences. Mr. Felix Wassermann, graduate student at the Department of Microbiology, New York University College of Medicine, completed here the research for his doctoral thesis.

We have continued to work in close co-operation with colleagues at the Biological Laboratory of the Long Island Biological Association. Members of the Laboratory research staff joined us in our staff meetings and took part in our program of seminar lectures. A major portion of the year-round research at the Laboratory deals with genetical problems related to our studies. During the past year B. Wallace and J. C. King continued their investigations of population genetics with *Drosophila*, E. Englesberg and P. D. Skaar worked in bacterial genetics, and H. Moser began research with human cells in tissue cultures.

We have also profited by association with visiting research workers at the Biological Laboratory. Among these were

S. Granick, R. D. Hotchkiss, and K. Maramorosch, Rockefeller Institute for Medical Research; A. Bernheimer, New York University College of Medicine; A. Novick, University of Chicago; S. E. Luria, University of Illinois; E. W. Caspari, Wesleyan University; Evelyn M. Witkin, State University of New York; E. Calef, University of Pavia; T. Watanabe, Keio University, Tokyo; M. Errera, University of Brussels; and R. W. Barratt, Dartmouth College.

Again this year Kaufmann and Gay received the co-operation of members of Brookhaven National Laboratory in their use of an electron microscope, and McClintock also utilized facilities at Brookhaven in connection with her work with maize.

#### MEETINGS AND LECTURES

The twenty-second Cold Spring Harbor Symposium on Quantitative Biology met at the Biological Laboratory for ten days in June 1957. It was attended by about 150 scientists interested in the fields of animal ecology and demography. The purpose of the meetings was to bring together outstanding scientists of these two fields as well as geneticists, anthropologists, economists, and statisticians, in order that the experimental and analytical methods and conclusions of each group might be shared by the others.

A conference of scientists engaged in research with bacterial viruses, organized by Hershey and Burgi, was held at the laboratories in August 1957, with about a hundred participants from this country and Canada.

Weekly meetings of the research staffs of the Department and the Biological Laboratory were held from October to May, for informal discussion of scientific problems of general interest and for reports of the current research of individual members. Seminar lectures were also scheduled each week throughout most of the year. They were attended by scientists from



near-by institutions as well as by the scientific members of the laboratories. The speakers, who included staff members, members of the summer group, and invited guests, presented reviews of completed research problems in which they had made major contributions.

#### OTHER ACTIVITIES

During the year, 1002 cultures of *Drosophila melanogaster* stocks were sent to high schools and colleges for use in the teaching of genetics. The requests for this service came from 41 states and from 4 countries outside the United States. Mrs. G. C. Smith continued in charge of the *Drosophila* stocks.

The thirtieth issue of *Drosophila Information Service*, with Demerec as editor, was prepared at the Department and distributed in January 1957. The Department continued to handle the preparation and distribution of *Microbial Genetics Bulletin*, which is compiled and edited by Dr. Evelyn M. Witkin, of the College of Medicine of the State University of New York. Number 14 of the *Bulletin* was issued in November 1956. The tenth issue of *Phage*

*Information Service*, containing abstracts of discussions presented at the August 1956 meeting of bacteriophage workers, was prepared at the Department under the direction of Burgi and issued in November 1956.

The library, with Mrs. G. C. Smith as librarian, acquired 436 books, of which 61 were purchased, 19 were received as gifts or exchanges, 331 were added as periodicals currently bound, and 25 were added from the Davenport Collection. Periodicals and serial publications received regularly numbered 369. Interlibrary loan service was received from Brookhaven National Laboratory, Columbia University, the Long Island Agricultural and Technical Institute, the Rockefeller Institute for Medical Research, and the University of Buffalo; and our interlibrary facilities were made available to Brookhaven National Laboratory, the California Institute of Technology, Chas. Pfizer & Co., the University of Connecticut, and the Veterans Administration Hospital in Tucson, Arizona. During the year, 319 books and periodicals were loaned to staff members, assistants, and guest investigators.

#### GROWTH AND INHERITANCE IN BACTERIOPHAGE

*A. D. Hershey, Elizabeth Burgi, Joseph D. Mandell, and Jun-ichi Tomizawa*

Phage T2 behaves in genetic experiments as though each particle contains a single set of linear chromosomes. According to recent experiments by Streisinger, there is in fact only one such chromosome. There is little doubt that this invisible but thoroughly familiar chromosome contains nucleic acid (DNA). In current work we are asking three questions about the chromosomal substance. Is it DNA exclusively? Is all the DNA in phage particles chromosomal DNA? Is the formation of chromosomal DNA in infected bacteria independent of the formation of phage protein?

Somewhat to our surprise, the answer to the first question is probably yes. The other questions are by no means answered,

but we, and others, seem to be coming to grips with them.

The work summarized below is partly supported by a grant (C-2158) from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service. Mandell is a Fellow of the Carnegie Institution of Washington.

#### *Composition of Chromosomes*

Phage-precursor DNA can be formed in the presence of chloramphenicol, which almost completely inhibits protein synthesis as measured by incorporation of radio-sulfur into acid-insoluble materials. In the course of work with various systems, Melechen found that chloramphenicol seldom permitted a normal rate of DNA

synthesis, though no inhibition had been noticed in earlier experiments by him or by others. His observation, perhaps not interesting in itself, prompted a search for particular phage proteins whose synthesis might be poorly suppressed by chloramphenicol and essential to synthesis of DNA, a search otherwise necessary in any event.

As a preliminary to this work, a systematic examination of particles of phage T2 was made in an attempt to identify as many minor components as possible, especially those that might associate with DNA. Only two new components were found: an acid-soluble peptide, composed chiefly of aspartic and glutamic acids and lysine; and "substance A," probably representing one or two unidentified free amino acids. Substance A can be regarded as a single substance in metabolic experiments, and is chiefly characterized by the fact that arginine is a major precursor. The peptide and substance A each contain about 1 per cent of the total carbon of phage particles.

When  $C^{14}$  arginine is fed to infected bacteria and the yield of labeled phage particles is isolated, only substance A, among acid-soluble constituents, is labeled. Similarly, from phage particles labeled with  $C^{14}$  lysine it is easy to separate the labeled acid-soluble peptide from other labeled substances. These facts have facilitated experiments yielding the following information.

DNA and substance A, but not lysine- or arginine-labeled protein or acid-soluble peptide, are transmitted from parental to offspring phage. Substance A is not a constituent of DNA, however, and its transfer from parental to offspring phage is of no genetic significance. Substance A is readily separable from DNA by dialysis if the phage particles are first disrupted by osmotic shock, and it is efficiently incorporated into phage particles if supplied to infected bacteria as a constituent of the culture medium.

These and other findings show that

phage DNA, and hence chromosomal DNA, are not permanently associated with more than about 1 per cent by weight of phage protein, if with any. Since chloramphenicol suppresses formation of total phage protein and of acid-soluble peptide about equally, it seems unlikely that any particular fraction of the protein is spared by the antibiotic. Our results are consistent with earlier work of Hahn, Wisseman, and Hopps in suggesting that chloramphenicol inhibits peptide synthesis generally and specifically.

### *Autonomy of Synthesis of Chromosomal DNA*

In earlier experiments with Melechen we found that phage-precursor nucleic acid (DNA) can be formed in infected bacteria whose capacity to form protein is blocked by chloramphenicol. By adding chloramphenicol 9 minutes after infection and removing it at 60 minutes, for instance, one can obtain phage particles containing almost exclusively the phosphorus of DNA synthesized before the sixtieth minute and protein synthesized after the sixtieth minute. A detailed analysis of this result by kinetic tracer experiments seemed to prove that synthesis of phage DNA and synthesis of phage protein are sequential processes (see Year Book 54, 1954-1955, pp. 216-219). This conclusion evidently calls for genetic tests for the accumulation of chromosomal DNA in the presence of chloramphenicol. Several such tests have been designed and are being applied. Only one of them has so far yielded interpretable results.

Tomizawa finds that if infected bacteria are irradiated with five phage-lethal doses of ultraviolet light at various times during the period of treatment with chloramphenicol, and phage particles are subsequently isolated after removal of the chloramphenicol, a constant number of phage particles is obtained of which a variable fraction is noninfective. The number of noninfective particles is roughly proportional to the



amount of DNA in the cells at the time of irradiation. If the amount is large, most of the particles formed are noninfective. The noninfective particles have all the properties, as far as these have been tested, of irradiated phage particles. They attach to bacteria, inject their DNA, and make genetic contributions to the offspring of mixed infection with live particles. These facts strongly suggest that phage-precursor DNA formed in the presence of chloramphenicol is functionally equivalent to the DNA in phage particles.

#### *Fractionation of Phage DNA*

A number of people are attempting to fractionate phage DNA by various physical and biological means, in the hope of recognizing functional diversity if it exists. Burgi and Mandell have made appreciable further progress along these lines.

On the physical side, they have modified the methylated serum albumin column of Lerman so that it now yields authentic fractions. So far these fractions have been identified only in terms of their behavior on the column itself. At least three fractions, on repeated test, elute from

the column at slightly different concentrations of sodium chloride. The relative amounts of these three fractions are different in whole phage particles and in the  $P^{32}$ -labeled DNA transmitted from parental to offspring phage. The transfer seems to enrich the fractions that elute from the column at lower salt concentrations. Unfortunately, this result is incomprehensible in terms of any of the existing ideas about mechanism of transfer. At any rate it cannot be interpreted until something has been learned about the chemical basis of the fractionation and the metabolic origin of the fractions.

#### *Conclusion*

The theory that the chromosomes of future phage particles multiply in the form of naked molecules of DNA in infected bacteria has been substantiated.

Preliminary work on the physical fractionation of phage DNA shows that labeled DNA transferred from parental to offspring phage consists of a characteristic fraction. At the moment this result merely suggests that existing ideas about the mechanism of transfer are inadequate.

### GENETIC STUDIES WITH BACTERIOPHAGE

*G. Streisinger, Marian Martinello, and F. Wassermann*

#### *Forward and Reverse Mutations*

Recent results of Benzer with bacteriophage, and results with other organisms, indicate that short regions of the genome act as units in the determination of the specificity of the product. Two mutant genomes do not complement each other, when present in the *trans* configuration within a common cytoplasm, if the sites of the mutations are in the same functional unit. A useful working hypothesis is that the deoxyribonucleic acid (DNA) code of the functional unit determines details of the configuration of a product protein. It would seem likely on this basis that mutations in one direction resulting in the loss of a function occur at any of a large

number of sites within a functional unit, whereas reverse mutations, resulting in restoration of that function, occur precisely at the site of the forward mutation.

We have examined this question by experiments with the  $h \rightleftharpoons h^+$  mutational system in the bacteriophage T2L, described previously by Streisinger and Dr. N. Franklin. Phage strain  $h_00$ , which adsorbs to bacterial strains B and B/2, gives rise to mutant  $h^+$  strains, which do not adsorb to B/2. The various  $h^+$  strains yield recombinants when crossed to one another, and the sites of mutation can be mapped within a short region of the phage genome. They belong to the same functional unit, as determined by means of a *cis-trans* test.

*Different classes of reverse mutants.* The various  $h^+$  (forward) mutants differ from one another in rate of inactivation at high temperatures. Reverse  $h$  mutants obtained from various  $h^+$  strains were compared with respect to rate of inactivation at 65° C; about 10  $h$  mutants from each of about 20  $h^+$  mutants were analyzed. Most  $h^+$  strains produced exclusively  $h$  mutants identical with  $h_0\theta$ . Each of two strains, however, produced several  $h_0\theta$ -like mutants and one  $h$  mutant more temperature sensitive than  $h_0\theta$ . One strain,  $h^{+37}$ , gave rise to three classes of reverse mutants very different from one another and all very different from  $h_0\theta$ .

*Recombination analysis of  $h$  mutants derived from  $h^{+37}$ .* Mutant  $h$  strains that are identical with  $h_0\theta$  with respect to temperature sensitivity produce fewer than  $10^{-4}$   $h^+$  recombinants (if any) when crossed to one another or to  $h_0\theta$ . The three dissimilar classes of  $h$  mutants derived from  $h^{+37}$  were crossed to one another and to  $h_0\theta$  in tests for recombination. The procedure was as follows: (1) The parents in the crosses were ultraviolet irradiated, to increase the frequency of recombination. Each parent received about 15 phage-lethal hits, a dose that is expected (according to results of  $h^+i \times h^+j$  crosses) to increase the frequency of recombinants by a factor of about 4. (2) A second generation of the progeny (produced with low multiplicity in order to avoid phenotypic mixing and resolve any heterozygotes into their progeny types) was added to B/2 cells in the presence of potassium cyanide. The mixture was centrifuged after a 45-minute adsorption period, during which 75 to 90 per cent of the  $h$  particles, but none of the  $h^+$  particles, became attached to the B/2 cells. After centrifugation, the pellet was discarded and the supernatant was treated with chloroform. (3) The supernatant was plated with a mixture of B and B/2 bacteria on a large number of plates, and incubated.

Most of the  $h^+$  progeny showed high rates of reversion to  $h$ , like most  $h^+$  mutants

of  $h_0\theta$ ; therefore, they were clearly not recombinants. The few stable  $h^+$  types recovered were backcrossed to  $h^{+37}$ . Recombinant  $h$  particles were found among the progeny of all but one of these crosses. Thus only one, if any, of the  $h^+$  progeny of the  $h \times h$  crosses was an  $h^{+37}$ -like recombinant; this one  $h^{+37}$ -like phage could have arisen by independent mutation. Since ultraviolet treatment is expected to increase the fraction of recombinants by a factor of about 4, the frequency of recombinants was less than the fraction of phage unadsorbed divided by 4 times the number of progeny phage observed (after adsorption), that is, less than  $2.5 \times 10^{-6}$  for each cross. The site of each reverse mutation is thus less than 0.0005 recombinational unit from the site of the original mutation.

*Two states of the functional unit.* A functional unit can exist in either of two states, which may be designated "competent" and "incompetent," depending on whether or not its product is effective. Our results indicate that mutation from the competent to the incompetent state can take place at a number of different sites, whereas the reverse mutation from incompetent to competent can occur at only one site. The competent configuration of the functional unit is apparently not unique, however, since a number of non-identical reverse mutations have been obtained.

#### *The Determination of Host-Range Phenotype*

Previous results obtained by Streisinger and by others indicated that the elements of the phage particle responsible for the specific adsorption of phage to host cells are produced in a pool. The specificity of the elements is determined by the  $h$  functional unit, but the elements become associated more or less at random with phage genetic material during the course of maturation. It was shown that at least two elements are associated with each phage particle, but the number may be large.



A method employed by Hershey has now been utilized to determine the number of elements per phage particle. Crosses were made between the temperature-resistant  $h_0\theta$  and the temperature-sensitive mutant  $h^+37$  at various multiplicities of infection. No measurable inactivation of  $h_0\theta$  phage is effected by incubation at  $54.5^\circ\text{C}$  for periods of time that leave fewer than  $10^{-3}$   $h^+37$  survivors. Three alternative models were possible.

2. If one resistant element per phage particle confers resistance, the fraction of resistant particles would equal  $p \times 1 - (1 - f)^n$ .

3. If the level of resistance depends on the ratio of resistant to sensitive elements per particle, intermediate levels of resistance would be expected, or, at any rate, the fraction of resistant particles in a set of crosses made with different multiplicities would not be consistent with either of the above-stated models.

TABLE 1. Fractions of Temperature-Resistant Particles among the Progenies of  $h_0\theta \times h^+37$  Crosses Made with Three Multiplicities of Infection

A. Multiplicity of $h_0\theta$ per bacterium.....	4.5	0.17	0.11
B. Fraction of $h$ in the progeny.....	0.33	2.3	0.005
C. Fraction of infected bacteria with at least one $h$ , $=p$ .....	0.96	0.89	0.1
D. Fraction of $h$ among the progeny of bacteria yielding at least one $h$ ( $B/C$ ), $=f$ .....	0.34	0.19	0.05
E. Fraction resistant expected among progeny if a particle with one sensitive element is sensitive, $=p \times f^n$ when $n=2$ .....	0.11	0.033	$2.5 \times 10^{-4}$
$=3$ .....	0.04	0.006	$1.2 \times 10^{-5}$
F. Fraction resistant expected among progeny if a particle with one resistant element is resistant, $=p \times 1 - (1 - f)^n$ when $n=2$ .....	0.55	0.31	0.01
$=3$ .....	0.68	0.42	0.014
$=4$ .....	0.78	0.51	0.019
G. Fraction resistant observed among progeny.....	0.55	0.23	0.01
H. Fraction resistant expected among $h$ progeny, $=1 - (1 - f)^n$ when $n=2$ .....	0.57	0.34	0.1
I. Fraction resistant observed among $h$ progeny.....	0.75	0.39	0.3

Note: Crosses were made with starved bacteria in buffer, the total multiplicity always being between 10 and 20. The fraction of bacteria infected with at least one  $h$  particle was determined by plating the infected bacteria, after serum treatment to remove unadsorbed phage, on a mixture of B and B/2; bacteria yielding  $h$  produced clear plaques. The infected bacteria were diluted in salt-free broth and, after a period of 2 hours at  $37^\circ\text{C}$ , were shaken with chloroform. The progeny phages were treated for 6, 12, or 18 minutes at  $54.5^\circ$ . The fractions surviving the three time treatments were very similar; the figures given are the means.

1. If one sensitive element per phage particle confers the level of sensitivity typical of  $h^+37$ , inactivation curves of the progeny of  $h^+37 \times h_0\theta$  should level off, owing to the presence of a fraction of resistant particles. This fraction of resistant particles would be equal to  $p \times f^n$ ,  $n$  being the number of elements per phage particle and  $f$  the fraction of resistant elements in the pool in the proportion,  $p$ , of bacteria infected with at least one  $h$  particle. It is assumed that  $f$  equals the fraction of  $h$  in those bacteria infected with  $h$ .

The fractions of resistant particles in the progenies of crosses with three different multiplicities are given in table 1. The results agree fairly well with what is expected on the basis that each phage particle has a few, most probably two, elements, resistance being conferred by the presence of at least one resistant element. The rate of inactivation at  $65^\circ$  was measured in progeny from two of the crosses. The ultimate slopes resembled the slope of a control  $h_0\theta$  population; the fraction of resistant progeny, determined by extrapolation

of the ultimate slope, was the same as that at 54.5°.

The calculated fraction of survivors among the progeny does not take into account the distribution of multiplicities from bacterium to bacterium; that the correction involved would be very small was indicated by a calculation based on the actual distributions for one of the multiplicities.

The fraction of resistant *h* progeny is larger than the fraction of resistant *h*<sup>+</sup> progeny, even after a correction for *h*<sup>+</sup> produced in bacteria infected exclusively with *h*<sup>+</sup> phage. This association of phenotype and genotype could be due to fluctuations in the clone size of the *h* minority parent and in the fraction of resistant elements from burst to burst, or else to spatial correlations of genetic material and adsorption elements within the bacteria. In the latter case, if there was nonrandom association of genotype and phenotype, there would also be a tendency for similar elements to associate with the same phage particle, and conclusions with respect to the number of elements would not be valid. The question might be investigated further by means of crosses in which maturation was prevented for an extended period by treatment with chloramphenicol. In such crosses less variation in clone size would be expected to result from very unequal multiplicities of infection, because of the absence of sampling during most of the latent period.

#### *Genetic Relationship of T5 and PB*

Partial exclusion of phage T2 by the related phage T4 appears to be associated with the presence of more glucosylated hydroxymethylcytosine in the deoxyribonucleic acid of T4 than in that of T2. It seemed of interest to examine partial exclusion in another system.

Wassermann has been studying the partial exclusion that is observed when T5 is crossed to a related phage PB. These species grow on the common host *Escherichia coli* strain B, but differ with respect

to serological specificity and range of other susceptible hosts.

Ability to Grow on Hosts					
	B	B/5	B/PB	S	FCb
T5 . . . . .	yes	no	yes	no	yes
PB . . . . .	yes	yes	no	yes	no

B/5 and B/PB are mutant derivatives of B to which the pertinent phages do not adsorb. S represents *Salmonella enteritidis*, and FCb is a strain of *E. coli*.

*Exclusion.* Two levels of exclusion were observed in T5×PB crosses. Fractions of the bacteria, which were multiply infected with both parents, yielded exclusively T5 or PB. The rate of adsorption of both parents is very low ( $K = \text{approx. } 4.0 \times 10^{-10} \text{ min}^{-1}$ ), and it is possible that this complete kind of exclusion is caused by superinfection breakdown.

The remaining fraction of mixedly infected bacteria gave both T5-like and PB-like phage, as determined by plating infected bacteria, after serum treatment, on a mixture of B/5 and B/PB. Single-burst experiments showed that most of the yield from these cells was like PB with respect to host range, whereas only a small fraction was like T5. There seems, therefore, to be a second kind of exclusion mechanism, which gives rise to unequal yields of markers from the two parents.

*Determination of levels of exclusion.* Progeny of T5×PB crosses carrying markers from T5 and PB were isolated from a number of single bursts. When crossed to the T5 parent, they all excluded T5 at least partially. Since the degrees of exclusion in these crosses varied with different hybrids, either when crossed to one another or when crossed to the T5 parent, it can be concluded that several factors control the levels of exclusion.

An attempt was made to incorporate markers from PB into an otherwise T5-like genome by crossing heavily UV-irradiated PB (about 200 hits) with unirradiated T5. Two different hybrids recovered from the progeny were crossed to T5. Both



hybrids partially excluded T5. It is assumed that only short sections of the genome of the irradiated PB parent were incorporated into the otherwise T5-like hybrid progeny. Exclusion of T5 by these hybrids suggests either that the number of factors responsible for exclusion is large or that the excluding factors are closely linked to the host-range markers.

*Control of the host-range differences between T5 and PB.* Hybrid phage recovered from PB × T5 crosses, or from crosses between hybrids, produced plaques on either B/5 or B/PB, but never on both. The markers responsible for these alternative host ranges thus appear to be allelic or at least very closely linked. The possibility that some recombinants are not detected because of the exclusion of alternative adsorption elements on the phenotypic level cannot be ruled out until phenotypic mixing is investigated. Most of the hybrid strains that formed plaques on FCb did so with lower efficiency than on B. The adsorption rate constant of these hybrids on FCb was the same as that of T5 on the same host ( $K = \text{approx. } 4 \times 10^{-9} \text{ min}^{-1}$ ); but only about one-tenth of the phage adsorbed yielded progeny able to form plaques on either B or FCb.

Among the hybrids recovered from

crosses of T5 with UV-irradiated PB, those that produced plaques on FCb did so with high efficiency. PB, therefore, seems to contain factors that affect the ability of T5 to grow on its specific host FCb. Such crosses also yielded hybrids able to form plaques on *S. enteritidis*. They did so with low efficiency, however, and adsorbed much more poorly on this strain than did PB. Adsorption to *S. enteritidis* thus appears to be subject to control by several factors.

Most of the hybrids recovered in all crosses formed plaques on either FCb or *S. enteritidis*, although often with low efficiency. A hybrid was recovered that would not form plaques or adsorb on either strain. It seems unlikely that this hybrid is recombinant, however, since no hybrids having the capacity to adsorb to both *S. enteritidis* and FCb could be recovered.

Since the hybrids were predominantly recombinants between the B/5-B/PB markers and the S-FCb markers, it may be inferred that these two pairs of markers are not closely linked.

The exclusions observed, as well as the paucity of additional markers, have prevented for the time being a more precise genetic analysis and mapping of the genomes of these related phages.

## BACTERIAL GENETICS

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Our group has continued to study genetic mechanisms, applying transduction and transformation techniques in experiments with *Salmonella typhimurium*. Analysis of five closely linked genes, representing one cystine and four tryptophan loci, has shown that three sections of a transducing fragment may be incorporated simultaneously into the bacterial chromosome, a process that would necessitate sextuple crossing over in the event that a cross-over type of mechanism operates in transduction. Cumulative evidence confirms the finding that in studies of bacterial auxotrophs abortive transduction is an effective index of whether or not two closely linked

similar mutants are allelic. Results of further inquiries into the mechanism of transduction suggest that a transducing fragment is not a randomly selected section of the bacterial chromosome. Experiments with transformation have again revealed the occurrence of mutation at a locus near the marker used for selection.

In addition to the workers named above, our group included during part of the year Dr. Royston C. Clowes, of the Wright-Fleming Institute of Microbiology, St. Mary's Hospital Medical School, London, who was a Fellow of the Damon Runyon Memorial Fund, and Dr. Etta Käfer, who was a Carnegie Institution Fellow. Mrs.

Jean W. McIntyre continued in charge of washing and sterilizing glassware, and Mrs. Emmy M. Snyder in charge of preparing culture media.

During the summer of 1957, Professor Joseph S. Gots, of the Department of Microbiology, School of Medicine, University of Pennsylvania, worked with us as a guest investigator. With the assistance of Dr. Jack Gregory, he conducted studies of purine-requiring mutants. Dr. Harold Baer, of the Department of Microbiology, School of Medicine, Tulane University, studied histidine-requiring mutants. As temporary assistants during the summer of 1957 we had with us Miss Barbara Beckwith, of the University of California; Mr. W. Dilworth Cannon, of Yale University; and Miss Phoebe Starfield, of Swarthmore College.

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### *Mechanism of Transduction*

The transduction technique is proving to be a very valuable tool for studies of the genetic structure of genes and of small regions of chromosome in bacteria. In the process of transduction a phage particle carries a fragment of bacterial chromosome from the bacterial cell in which it grew into another cell which it infects. Genetic material from this fragment may be incorporated into the chromosome of the offspring of the recipient bacterium (completed transduction), or, more frequently, the fragment remains free in the cell (abortive transduction). Evidence discussed in previous reports indicates that in completed transduction parts of the bacterial chromosome are replaced by homologous parts of the transducing fragment, in other words, that transduction is accomplished through a process similar to crossing over. In abortive transduction the imported fragment does not participate in division of the recipient cell or formation of the new chromosome, but is passed on intact to one of the two daughter cells. Abortive transduc-

tion is detected through the appearance of minute colonies on minimal-medium platings of nutritionally deficient recipient bacteria infected with phage from bacteria carrying the wild-type allele of the gene responsible for the deficiency.

During the past year, Ozeki, extending our studies of the transduction mechanism in *Salmonella typhimurium* has obtained evidence that the chromosome fragment which a phage particle picks up from a donor bacterium and deposits in a recipient bacterium is not a randomly selected fragment. His findings suggest the possibility that the chromosomes of donor bacteria may be partitioned during the lytic process into small sections, in some regular way and at predetermined locations, producing for any one region uniform transducing fragments. The evidence of nonrandom transducing fragments is derived from three sets of experiments, which can be summarized as follows.

1. *Abortive transduction in double mutants.* Previous studies had revealed that four tryptophan loci (*tryA*, *B*, *C*, *D*) and one cystine locus (*cysB*) are so close together on the chromosome that all five may be included in one transducing fragment. The order of their arrangement was shown to be *tryD-tryC-tryB-tryA-cysB*. In Ozeki's experiments to elucidate the transduction mechanism, *tryD-10 cysB-12* bacteria were transduced with phage grown (a) on bacteria carrying the wild-type genes for both loci (*tryD-10<sup>+</sup> cysB-12<sup>+</sup>*), (b) on bacteria carrying the wild-type gene at one locus and the mutant at the other (*tryD-10 cysB-12<sup>+</sup>* or *tryD-10<sup>+</sup> cysB-12*), and (c) on bacteria carrying mutant genes at both loci (*tryD-10 cysB-12*). The infected bacteria were plated on "minimal" medium (see table 2), on minimal medium containing tryptophan (MT), and on minimal medium containing cysteine (MC). The data are given in table 2. The minute colonies represented abortive transductions in which the transducing fragment carried the wild-type allele (or alleles) for the deficiency (or deficiencies)



not satisfied by the selective medium used; and the large colonies represented completed transductions by the same classes of fragments, accomplished when the wild-type gene in the fragment replaced the mutant gene in the recipient chromosome.

The fact that similar numbers of minute colonies appeared on all plates when the donor was *tryD*<sup>+</sup> *cysB*<sup>+</sup> suggests that all the fragments responsible for these abortive transductions carried both *tryD* and *cysB* markers—that is, extended beyond *tryD* at one end and beyond *cysB* at the other. The presence of fragments carrying only one of the markers, resulting from a break

great as or greater than the distance between *tryD* and one end of a fragment or between *cysB* and the other end.

Additional evidence for the assumption that transducing fragments are nonrandom is derived from data involving the *athD* and *athB* loci, which also are carried in a single transducing fragment and located close together. In experiments with *athD-12* as recipient and *athB-6* as donor, minute colonies were considerably smaller than in experiments with *athD-12* as recipient and the wild type as donor. Since the frequency of appearance of minute colonies was not significantly different in the two

TABLE 2. Abortive Transduction in *tryD-10 cysB-12* Bacteria

Figures represent the numbers of minute colonies per plate obtained after 18 hours of incubation at 37° C (figures in parentheses, the numbers of large colonies resulting from completed transduction). "M"=minimal medium plus 1 mg/ml Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>; Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> is used to suppress the accumulation of tryptophan by the *cysB* factor of the recipient bacteria, and this medium may be considered "minimal" for the present experiment. MT=minimal medium plus tryptophan (20 µg/ml). MC=minimal medium plus cysteine (20 µg/ml).

Medium	Donor			
	<i>tryD</i> <sup>+</sup> <i>cysB</i> <sup>+</sup>	<i>tryD-10 cysB</i> <sup>+</sup>	<i>tryD</i> <sup>+</sup> <i>cysB-12</i>	<i>tryD-10 cysB-12</i>
"M" .....	4700 (122)	0 (0)	0 (0)	0 (0)
MT .....	4300 (601)	4100 (399)	0 (0)	0 (0)
MC .....	4000 (355)	0 (0)	2350 (135)	0 (0)

at some point between these two loci, would have increased the number of abortive transductions on the MT and MC plates, since the experiments showed that minute colonies appeared on MT plates when the donor was *tryD-10 cysB*<sup>+</sup>, and on MC plates when the donor was *tryD*<sup>+</sup> *cysB-12*.

Similar results could be expected, it is true, if the chromosomes of donor cells were divided at random into fragments that were always long in comparison with the distance between the *tryD* and *cysB* markers. This explanation is made unlikely, however, by the results obtained in extensive studies of recombination involving the *tryD*, *C*, *B*, *A*, and *cysB* loci, made by Demerec and Z. Hartman (Carnegie Inst. Wash. Publ. 612), which indicate that the distance between *tryD* and *cysB* is as

sets of experiments, and size distribution curves were unimodal in both, the inference is that every transducing fragment carrying the *athD-12* site also carried the *athB-6* site.

2. *Reciprocal transduction.* Reciprocal experiments with the linked loci *athC* and *gua* and also with *tryB* and *cysB* showed significant and consistent differences in ratios of one-marker to two-marker transductions. The results of experiments with *athC-5* and *gua-1* are given in table 3. The unlinked marker *pro-46* was introduced into both these mutant strains, to serve as an indicator of transduction frequency and assure quantitatively comparable data for the evaluation of reciprocal tests. These data show that, whereas incorporation of a portion of transducing fragment carrying both *athC-5* and *gua-1*

occurred in the two reciprocal experiments with about equal frequency, incorporation of *guA-1* singly was almost four times as frequent as incorporation of *athC-5* singly. Similar results were obtained in experiments involving *tryB-2* and *cysB-12*. Such ratios can be explained on the supposition that the transducing fragments were short, their ends were predetermined, and *athC-5* (or *tryB-2*) was located nearer to one end than *guA-1* (or *cysB-12*) to the other. According to this explanation, the position of a site on the fragment has an important effect on the frequency of transduction of the marker. Figure 1 is a schematic representation of the positions of the *athC-5* and *guA-1* sites on the transducing fragments, based on the values presented in

table 3. In class c of the table, the similar values obtained for the reciprocal experiments 1 and 2 are explained on the basis that in both cases the X and Z portions of the fragment were involved in the incorporation process; and the difference in values in class b is explained on the basis that experiment 1 involved the X and Y portions whereas experiment 2 involved Y and Z.

If the above reasoning is correct, the ratio of frequencies (*f*) of transduction to prototrophy in *athC-5* and *guA-1* with a wild-type donor may be expressed as  $f\text{ }athC/f\text{ }guA = X(Y + Z)/Z(X + Y)$ . The values of X, Y, and Z may be estimated from the b/c ratios in table 3, as follows:  $Y/Z = 0.93$ , and  $Y/X = 3.29$ ; accordingly,

TABLE 3. Reciprocal Transduction Experiments with Linked Markers

Each figure represents the actual number of colonies on a total of three plates. Figures in parentheses are adjusted values (per 1000 colonies in column a). (a) Transduction from *pro-46* to *pro+*. (b) Single-marker transduction: expt. 1, from *athC-5 guA+* to *athC+ guA+*; expt. 2, from *athC+ guA-1* to *athC+ guA+*. (c) Double-marker transduction: expt. 1, from *athC-5 guA+* to *athC+ guA-1*; expt. 2, from *athC+ guA-1* to *athC-5 guA+*.

Expt.	Recipient	Donor	a	b	c	b/c
1.....	<i>athC-5 guA+ pro-46</i>	<i>athC+ guA-1 pro+</i>	1072 (1000)	167 (156)	182 (169)	0.93
2.....	<i>athC+ guA-1 pro-46</i>	<i>athC-5 guA+ pro+</i>	2149 (1000)	1315 (612)	399 (186)	3.29

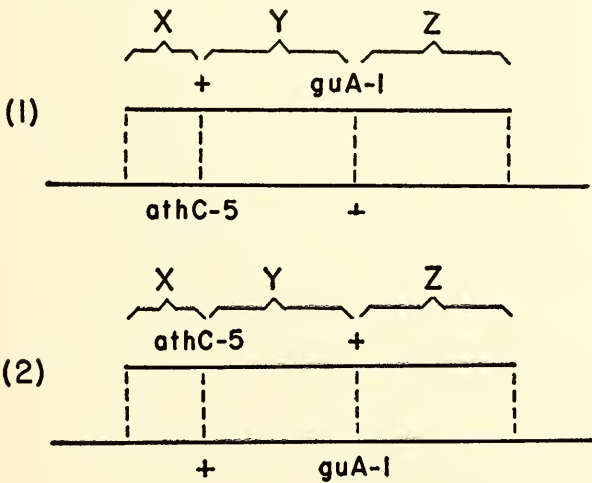


Fig. 1. Schematic representation of locations of genetic markers in the *athC-5-guA-1* reciprocal transductions. (1) and (2) correspond to experiments 1 and 2 in table 2. Lower line represents the bacterial chromosome, upper line the transducing fragment. X, Y, and Z: relative distances between genetic markers and terminal points on the transducing fragment.

$X/Y/Z = 0.93/3.05/3.29$ ; and  $f\text{ }athC/f\text{ }guA = 1/2.23$ . Ratios of transduction frequencies obtained in experiments fell within a range that agreed well with this estimation. The agreement between estimated and experimental values lends further support to the hypothesis that the ends of transducing fragments are predetermined.

3. *Frequencies of abortive and completed transductions.* A third source of evidence favoring predetermined transducing fragments was a comparison of frequencies of abortive and completed transductions in experiments with eleven auxotrophic strains as recipients and the wild type as donor. Pertinent data from the study are given in table 4. They make evident the following points. (1) Frequencies of abortive



transduction differed greatly among different unlinked gene loci, but the frequencies for closely linked loci, which can be transduced simultaneously (*adC-athC-guA* and *se-1-se-5*), were very similar. In other words, transduction frequency values are region specific. (2) The frequencies of completed transduction also differed greatly among unlinked loci; but the value for *adC*, which is presumed to be located near one end of a transducing fragment, was lowest among the values for the three linked markers, indicating that position on the fragment is one factor controlling transduction frequency. (3) The ratios of abortive to completed transductions showed

supplemented with indole or tryptophan; and *tryD* only on medium supplemented with tryptophan. Some mutants can also be recognized by determination of the substances they accumulate: *tryD* bacteria accumulate indole, and are able to feed *tryC*, *tryB*, and *tryA* bacteria; *tryB* accumulate anthranilic acid, and are able to feed *tryA*. Thus, on the basis of their requirements and accumulation products, *try* mutants can be readily identified when they appear singly. Certain combinations of the mutants, as, for example, *tryD tryC* or *tryD tryB*, can be distinguished in the same manner. Finally, it is possible to identify all other combinations of these

TABLE 4. Frequencies of Abortive and Completed Transductions of Eleven Auxotrophs by Wild-Type Donors

AT/P=number of abortive transductions per  $10^8$  phage particles; CT/P=number of completed transductions per  $10^8$  phage particles; AT/CT=ratio of abortive to completed transductions. Markers enclosed by brackets represent two groups of closely linked loci.

	Recipient										
	<i>hiD-39</i>	<i>athA-4</i>	<i>pro-46</i>	<i>cysA-1</i>	[ <i>adC-7</i>	<i>athC-5</i>	<i>guA-1</i> ]	[ <i>se-1</i>	<i>se-5</i> ]	<i>me-50</i>	<i>athD-12</i>
AT/P .....	4720	1790	845	425	280	310	250	360	315	225	120
CT/P .....	497	166	188	47	20	25	40	40	38	16	6
AT/CT .....	9.5	10.8	4.7	9.0	14.0	12.4	6.3	9.0	8.5	14.0	20.0

a considerably greater degree of uniformity than the transduction frequencies themselves. All these observations are in agreement with the theory that transducing fragments are predeterminate.

#### Recombination Studies

As was pointed out earlier, the four known tryptophan loci and one of the cystine loci are so closely placed that all five are included in one transducing fragment, and their order on the chromosome is *tryD-tryC-tryB-tryA-cysB*. Mutants representing the different *try* loci may be distinguished according to the compounds they require for growth: *tryA* bacteria grow on minimal medium supplemented with either anthranilic acid, indole, or tryptophan; *tryB* and *tryC* on medium

mutants by applying transduction tests, which are very sensitive although also very laborious.

In this material, therefore, we have available five good genetic markers, located close together on the chromosome, for studies of frequencies of recombination in transduction experiments. During the past year Goldman and Demerec have devoted much time to the preparation of material for such experiments, using the transduction process to build stocks that carry these markers in appropriate combinations.

At present we have only the data of one preliminary experiment, in which the recipient bacteria had the genetic constitution *tryD-10 tryB-4* and the donor strain was *tryC-3 tryA-8 cysB-12*. Potentially, transductions between these groups of markers could produce recombinants in-

volving exchanges in six divisions of the *try-cys* region, as follows:

—(1)—*tryD-10*—(2)—*tryC-3*—(3)—*tryB-4*—(4)—*tryA-8*—(5)—*cysB-12*—(6)—

Only those classes of recombinants that could be scored easily were fully identified. The frequencies and genotypes of identified recombinants, and the sections in which exchanges must have occurred to produce them, are given below:

2874	<i>tryA-8</i>	1-2-3-5
641	+	1-2-3-4
1476	<i>tryA-8 cysB-12</i>	1-2-3-6
22	<i>cysB-12</i>	1-2-3-4-5-6

The most significant implication of these results is the existence of recombinants requiring exchange in each of the six divisions. If exchanges in transduction are accomplished by a process similar to crossing over, these recombinations would correspond to sextuple crossovers.

#### *Abortive Transduction*

Abortive transduction brings about duplication of a small region of bacterial chromosome, which persists for a number of cell divisions, thus permitting the study in *trans* configuration of genes located in the duplicated region, and providing a very sensitive test for allelism among similar, closely linked mutants. A method applying this test to auxotrophic mutants of *S. typhimurium* was developed two years ago by Ozeki (Year Book 55, pp. 302-303), and since that time sufficient evidence has accumulated to indicate that it has general validity.

To permit detection of the minute colonies that represent abortive transductions, the medium on which recipient bacteria are plated should allow some background growth and at the same time favor growth of the minute colonies. Therefore it is desirable, and sometimes essential, to supplement a minimal medium with certain compounds. As a rule, studies of abortive transduction are facilitated by enrichment of the minimal medium with a small amount of broth. In the case of *tryD* mu-

tants, the size of minute colonies is markedly increased by supplementation with

0.02 to 0.05 per cent of neutralized vitamin-free casein hydrolysate; and in the case of *adC*, *adE*, and *adF* mutants, the addition of casein hydrolysate (0.1 per cent) is necessary for detection of the colonies. In work with *athA*, *athC*, and *athD* bacteria, detection is facilitated by supplements of adenine and pantothenate.

Abortive-transduction tests have now revealed that the mutants originally considered to be alleles of the *hiA* locus actually belong to three separate loci, and that the previously designated *cysA* and *adE* groups can each be divided into two groups. Genetic analyses of other groups of mutants have also been considerably advanced by means of abortive-transduction techniques.

#### *Analyses of Auxotrophs*

*Purine mutants.* Additional studies of purine-requiring mutants have been made by Dr. J. S. Gots with the assistance of Dr. Jack Gregory. They have provided further evidence that two groups of adenine-requiring mutants, *adE* and *adC*, are indeed genetically distinct, although phenotypically indistinguishable, by demonstrating the occurrence of abortive transduction between the two groups. Detection of the minute colonies resulting from abortive transductions was made possible by the addition of casein hydrolysate (0.1 per cent) to the plating medium.

The use of casein hydrolysate also made it possible to demonstrate abortive transduction between several members within the *adE* group. Preliminary results indicate that *ad-6*, *-8*, *-15*, and *-19* are functionally different from other mutants of the previously designated *adE* locus.

A mutant was isolated from *adE-11* that can grow as well on casein hydrolysate as on adenine or any of the other purines. This strain accumulates the same aminoimidazole as its progenitor, but can grow



slowly on minimal medium with 3 to 4 days of incubation. Its growth is stimulated by histidine, and to a lesser extent by glycine, methionine, arginine, and aspartic acid. In transduction tests, phage grown in bacteria of this strain was able to transfer the alternative amino acid requirement to all members of the group *adE*, including the four members named above that could be separated from the others by abortive-transduction tests. Members of the *adC* group were transduced only to wild-type prototrophs. The current interpretation of the observed properties is that this mutant represents a suppressor mutation of *ad-11*, which can act as a general suppressor for the entire *adE* group. The possibility that it may represent a different allele of *adE* has not yet been ruled out.

An analysis of those adenine-thiamine-requiring mutants that can grow on medium containing pantothenate or methionine instead of thiamine has revealed that, except in strains *ath-8* and *-19*, the requirement for thiamine is not completely satisfied by these chemicals. Pantothenate and methionine apparently reduce the amount of thiamine required, so that the small amount present in minimal medium enriched with broth is adequate for growth. Quantitative studies with *ath-6* showed that 0.0002  $\mu\text{g}$  of thiamine per ml gave half-maximal growth. Pantothenate or methionine reduced this requirement to less than 0.00002  $\mu\text{g}$ .

For *ath-8* and *ath-19*, it is known that addition of pantothenate, methionine, or valine to the medium can reduce the thiamine requirement to zero. When xanthine is the source of purine no additional factor is necessary for growth. These facts suggested that the requirement in the presence of adenine might be an expression of the known inhibitory action of adenine on growth of the wild type. This inhibition was studied with the wild-type strain LT-2. As little as 40  $\mu\text{g}$  of adenine per ml can prevent growth of this strain, but thiamine (0.1  $\mu\text{g}/\text{ml}$ ) completely removes the inhibition. The thiamine requirement of *ath*

strains, however, can be explained only in part by the action of thiamine in preventing inhibition by adenine. Histidine, which can relieve adenine inhibition, cannot replace thiamine as a growth factor for the *ath* mutants. Furthermore, an adenine-resistant strain of LT-2 was unable to transduce the *ath* strains to thiamine independence.

When *ath* strains are plated on medium lacking thiamine, occasional colonies grow out which feed the background mutant, as is evidenced by formation of satellites. Such feeder colonies were isolated from *ath-1*, *-3*, *-6*, *-7*, *-8*, *-9*, *-16*, *-19*, and *-20*. By testing with *Escherichia coli* auxotrophs having specific deficiencies in thiamine synthesis it was possible to show that the substance excreted by the feeder strains did contain thiamine. In the one exception among 12 strains examined, the feeding was due to excretion of the pyrimidine moiety of thiamine. All the *ath* strains can use the pyrimidine moiety, but not the thiazole moiety, for growth. All the feeder strains were used as donors in transduction tests with the original *ath* mutants, but repeated attempts to transfer either the feeding property or thiamine independence were unsuccessful.

*Cystine mutants.* Howarth has made an extensive study with eight cystine-requiring mutants originally considered to be alleles of a single locus, *cysA*. Abortive-transduction tests revealed that two closely linked loci are represented in this group, now designated *cysA* (*-1*, *-3*, *-13*, *-21*, *-69*, and *-82*) and *cysF* (*-22* and *-32*). Further tests showed that the previously described multisite mutant *cys-20* "covers" all the known sites of both *cysA* and *cysF*.

Howarth also isolated and analyzed eight independently originating suppressor mutations, two occurring in the multisite mutant *cys-20* and six in single-site mutants of the *cysA* and *cysF* groups. Her studies showed that at least five and probably all eight of these suppressors are nonspecific, and suppress both the multisite mutant *cys-20* and all known single-site mutants

of both loci. Such a finding was anticipated with respect to suppressors originating in the multisite mutant, for it seemed likely that they would be capable of suppressing all other alleles located in the region of the chromosome covered by this probable deletion. It was expected, however, that some of the suppressors isolated from single-site mutants might be specific. Although no biochemical analysis of the suppressor action has been attempted, it seems likely that the function of a suppressor operative for a multisite mutant as well as for all known single-site mutants of the same region is the introduction of an alternative pathway for the synthetic step blocked in the original mutant.

It had previously been noted that *cysB* mutants can feed all the known tryptophan-requiring mutants; this suggested that *cysB* cells accumulate and excrete tryptophan. Supporting evidence has been obtained by Gots and Gregory in experiments summarized as follows. Nonproliferating cell suspensions of *cysB-12* were incubated in minimal salts-glucose medium for 6 hours, with aeration, and the cells were removed by Seitz filtration. Tests showed that the filtrate was able to support partial growth of the tryptophan-requiring mutant *tryD-10*. After concentration by vacuum distillation, the filtrate was chromatographed on paper with butanol-acetic acid-water (40:10:10) as the solvent. Chromatograms were also made with a similar filtrate from a *cysD* mutant (*cysD-36*) that does not feed *tryD*. The *cysB* chromatograms showed five separate ninhydrin-positive spots. One of them was identified as tryptophan, and two corresponded to spots found in the *cysD* chromatograms. When eluates from the paper were tested for feeding of *tryD*, two different feeding substances were found. One was at the location of tryptophan, and the other, at a higher *R<sub>f</sub>* level, was ninhydrin negative. Thus, four substances are excreted by *cysB* that are not excreted by *cysD*. One is tryptophan; two are unidentified compounds that do not

feed *tryD*; and the fourth is a ninhydrin-negative substance that does feed *tryD*. It has been observed that substances supporting the growth of *tryD* mutants are not excreted by *cysB* if the medium contains either cysteine or a high concentration of thiosulfate (1 mg/ml).

### Transformation

Two years ago (Year Book 54, pp. 230-231) we reported apparent success in bringing about transformation in *Salmonella typhimurium*, that is, the transfer of genetic markers from the wild-type strain LT-2 to several mutant strains through the medium of disintegrated bacteria of the donor type or of DNA extracted from them. Such results have been obtained only in occasional experiments, however, and a number of technical problems remain to be solved before the mechanism of the process can be interpreted.

As was reported previously, when the recipient bacteria carried a *tryD* marker, we frequently detected mutations at the *tryC*, *tryB*, *tryA* loci, which are in proximity to the marker locus. During the past year a detailed analysis of this observation has been carried out by Demerec, Lahr, and Goldman. In the particular experiment made, the recipient bacteria carried the *tryD-7* marker, and the donor bacteria were of the wild type. The procedures were similar to those followed in transformation experiments with pneumococcus. The donor bacteria were grown in synthetic medium, the cells were lysed by freezing and thawing, and DNA was extracted by the Sevag method. Recipient cells were grown in aerated broth medium to the late log phase (about  $2 \times 10^8$  cells per ml), and a  $1 \times 10^{-5}$  dilution was made. Equal portions of this dilution and of a  $1 \times 10^{-2}$  dilution of DNA were mixed, and 0.1-ml samples of the mixture were plated on minimal agar enriched with broth (0.01 per cent broth powder).

A total of 630 colonies was obtained on three plates (218, 210, 202). Control plat-



ings of similar numbers of bacteria without DNA did not produce any colonies. When colonies from one plate were replicated on minimal, minimal-plus-indole, and minimal-plus-anthranilic acid media, they grew on the indole but not on the other two. This test showed that the cells of these colonies were either *tryB* or *tryC*, but not *tryA*, which can grow on anthranilic acid, or *tryD*, which cannot grow on indole. Thus it was revealed that the colonies differed in genetic constitution from both the recipient bacteria (*tryD*) and the donor bacteria (wild type); and it seemed likely that in this experiment one or more new tryptophan mutants were in some way induced.

Several further tests were made with 47 strains derived from colonies of the two plates not used in replica plating. All strains that were tested for accumulation of anthranilic acid did accumulate it, thus displaying one of the characteristics of mutants of the *tryB* locus. Four strains were transduced with representative markers of the *tryA*, *tryB*, and *tryC* loci (*tryA*-8; *tryB*-2, -4, -16; *tryC*-3). Large numbers of recombinants were obtained with *tryA*-8 and *tryC*-3, small numbers with *tryB*-16, and very small numbers with *tryB*-2 and *tryB*-4—an indication that these four strains represented mutation at the *tryB* locus, and that the site or sites of mutation were located close to the sites of *tryB*-2 and *tryB*-4. Transduction tests were then made between five of the strains as recipients and another four as donors, and also between 46 of the strains as recipients and one (strain 16) as donor. The fact that no recombinants were detected showed that the same *try* mutation was represented in all 47 strains. Twelve strains were transduced with all alleles of the *tryB* locus

known at the time (*tryB*-2, -4, -12, -13, 14, -16, and -19); and recombinants appeared in every case, showing that the new mutant was different from any other *tryB* mutant then available. From the frequencies of recombination it could be inferred that the site of mutation was closer to *tryB*-4 and *tryB*-2 than to any of the other allelic sites. Moreover, transduction tests with the diauxotroph *tryB*-2 *cysB*-45 resulted in the expected proportions of recombinants involving the three markers—in other words, normal recombination behavior for the *try-cys* region of the chromosome.

The new mutant was assigned the symbol *tryB*-26.

Thus this experiment, in which *tryD*-7 bacteria were combined with DNA extracted from wild-type bacteria, resulted in a large number of variants carrying both the wild-type allele of *tryD*-7 and a new mutant allele at the *tryB* locus, which is close to the *tryD* locus. Such variants were not observed in control platings of *tryD*-7 bacteria without DNA. A similar occurrence—that is, a change to wild type in the marker used for selection, and coincidental evidence of a mutation at a locus near the marker locus—had been observed in several earlier experiments. The case described here, however, was the first to receive extensive analysis. Tentatively, the term “transformation” is being used to designate the phenomenon, with no intention of implying that the responsible mechanism is identical with that operating in pneumococcus transformations. Unfortunately, we have not been able to carry these investigations further. Although attempts to reproduce the original results have occasionally been successful, several experiments designed for further testing have failed to produce variants.

## GENETICS OF *Aspergillus*

Etta Käfer

From diploid, heterozygous strains of *Aspergillus nidulans* the following types of mitotic segregants are isolated: (1) diploid

segregants, resulting from mitotic crossing over; (2) haploid segregants; and (3) so-called “nondisjunctional” diploid segre-

gants. Very little is known about the processes that lead to formation of the last two types of segregants. Incidental information was obtained during an analysis of mitotic crossing over, carried out in the Department of Genetics at the University, Glasgow, Scotland. It was found that aneuploids, that is, haploids disomic for one or more chromosomes, play an important role as intermediate products in haploidization.

In a further analysis of the process of haploidization, undertaken at this Department, it was attempted to isolate such aneuploids from a well marked diploid strain, in which markers on all chromosomes made it possible to determine the number of disomic chromosomes and to observe the breakdown of the unstable aneuploids into haploids.

Two haploid stocks, each containing markers of six linkage groups, were used to synthesize a multiply heterozygous diploid, which carried markers of linkage groups I and II on both arms of both homologous chromosomes, at least one marker of linkage groups III, IV, and V on each homologous chromosome, and one marker each of linkage groups VI and VII. Pure cultures of aneuploid segregants were obtained by means of the plating techniques described below.

Among the green conidial heads of the parental diploid are found yellow and white heads, which represent segregants that are homozygous or hemizygous for the color markers  $y$  and  $w$ . Conidia from these segregants were removed by touching the conidial heads with a fine needle. The conidia sticking to the needle were rinsed off in 0.2 ml of saline with a wetting agent added. The conidial chains were broken up by vigorous shaking, and the suspension was poured onto one plate of complete agar medium (CM). The number of pure colonies obtained in this way from each segregant varied from 1 to about 50.

In approximately 200 such platings, about 50 per cent of the segregants were found to be diploid ( $y/y$  or  $w/w$ ), about 40 per

cent aneuploid, and 10 per cent haploid. Aneuploid segregants could be recognized by their phenotype. They grew slowly, and had poor conidial formation and usually a dark background color. Contrary to expectation, at low density the viability of conidia from the aneuploids was almost 100 per cent. All aneuploid colonies produced occasional haploid sectors, which grew much more rapidly and vigorously.

If the number of such haploid sectors was small on the original plate, the aneuploid was replated at low density on CM. Aneuploid centers and haploid sectors (10–20 of each type) were then tested for requirements. It was found that markers of some linkage groups segregated in the sectors, whereas the aneuploid centers did not express these markers because they were heterozygous for them. It could therefore be determined for which chromosomes the aneuploid was disomic. The maximum number of disomic chromosomes found in these yellow and white segregants was three; most of them were disomic for just one chromosome.

Aneuploids could be maintained—that is, the formation of haploid sectors could be inhibited—by plating the aneuploid on a medium that selected against the markers carried on the disomic chromosomes. Intermediate steps in the breakdown to haploidy could be obtained by plating on appropriate selective media. When selection was applied against the markers of only one disomic chromosome pair, the original aneuploid formed sectors that segregated for one or two chromosomes but were still disomic for the selected chromosome. These aneuploid sectors ( $n+1$ ) formed haploid sectors when plated on CM.

The frequency of disomy was found to be not the same for all chromosomes. The chromosomes of linkage groups III and IV remained disomic most frequently.

As a second method of obtaining aneuploid colonies, large numbers of conidia of the original diploid were plated on CM. Several thousand colonies were inspected



for the aneuploid phenotype, and all possible cases were replated. In contrast to the earlier method, this procedure did not select for haploidy of any of the chromosomes. Aneuploids containing larger numbers of disomic chromosomes were isolated. As a maximum, an aneuploid was found which segregated for six of the marked chromosomes.

These results showed that most haploids arise as a consequence of a stepwise loss of one member of the various chromosome pairs. Of the intermediate aneuploid stages, those with a large number of disomic chromosomes seem to be less viable, whereas aneuploids with only one disomic pair have very good viability. Even these aneuploids, however, are outgrown when in competition with the derived haploid.

In two cases, "nondisjunctional" types were obtained as well growing sectors from poorly sporulating centers, which also yielded sectors of the parental (prototrophic) phenotype. A possible explanation is that the center strain was trisomic for one chromosome pair and diploid for

the rest. Two types of sectors would then be formed, depending on which of the three homologous chromosomes was lost. These findings indicate that at least occasionally formation of "nondisjunctionals" is connected with aneuploid formation and probably is not due to double crossing over across the centromere.

Because in haploidization whole chromosomes are lost or retained, markers of the same chromosome always show complete linkage. Analysis by means of haploidization is therefore employed to detect linkage and to establish linkage groups. Evidence of seven linkage groups had been obtained in previous research at the University in Glasgow. When several new markers were tested for linkage in mitotic haploids, three showed no linkage with any of the seven marked chromosomes but total linkage with one another; and an eighth linkage group was therefore established. The demonstration of eight linkage groups is consistent with recent cytological evidence of eight chromosome pairs in *A. nidulans* at meiosis.

## THE NATURE OF THE MATERIALS OF HEREDITY

*B. P. Kaufmann, Helen Gay, Deepesh N. De, and Yoko Yoshida (Cytology)*  
*Margaret R. McDonald and Randi Spømme (Chemistry)*

The activities summarized in this report have again been focused on analyses of patterns of organization of hereditary materials. The need for continuation and extension of this type of research has been re-emphasized during the past year by a growing awareness throughout the world of the genetic hazards of ionizing radiations. It has become increasingly apparent that the extensive quantitative data heretofore accumulated about kinds and degrees of degradational changes produced by radiations on the chromosomes represent only the first approach to an understanding of the nature of radiation damage, and that they must now be supplemented, if basic mechanisms are to be identified and palliative measures established, by exacting analyses of the alterations produced in the patterns of association and function of

cellular materials. Our earlier work with X rays and chemical agents led us several years ago to a consideration of methods for modifying the effect of radiations on the structure and function of chromosomes in the cells of higher plants and animals. Continued pursuit of these aspects of cellular morphology and physiology now finds greater incentive in the world-wide demand for more basic information about the action of ionizing radiations, particularly with reference to the genetic endowment of the human species.

Throughout the development of this program, our work has been supported by the National Institutes of Health of the Public Health Service, and it is a pleasure to note that our investigations during the past year have again been facilitated by a USPHS research grant (RG-149).

Our research efforts this year have followed two main channels of inquiry, utilizing the high level of resolution of cellular fine structure afforded by the electron microscope. In one, we have attempted to combine methods of enzymatic hydrolysis, developed for studies with the ordinary light microscope, with modern techniques of electron microscopy, in order to determine the changes effected by radiations and chemicals in the finer structure of chromosomes. In the other, we have continued our investigations of the interactions of nuclear and cytoplasmic materials in plant and animal cells under various experimental conditions. In a third approach, planned co-operatively with our colleague, Dr. Hermann Moser, of the Biological Laboratory, we have initiated studies using chemically defined media for the culture of cells and organs, particularly those of genetically well analyzed insects such as *Drosophila melanogaster*, in efforts to reach by direct observation an understanding of the action of mutagenic agents on the living cell, preparatory to an analysis of the changes effected in the finer patterns of organization. Since the last-mentioned studies are in their preliminary phases, discussion of the findings will be postponed until a later report.

In the studies with the electron microscope we have been privileged to use the RCA instrument at Brookhaven National Laboratory, and four of our group (Gay, Yoshida, De, and Kaufmann) have been appointed guest investigators for this purpose. We are indebted to Dr. Howard Curtis, Director of the Biology Department, for permission to use the microscope, and to Dr. John Bergeron and Mr. Mark Gettner for their personal attention and assistance in its operation.

We have also had the opportunity in recent months to re-evaluate experimentally some conflicting interpretations about the structure of giant chromosomes of the Diptera, in co-operation with Professor Atif Sengün, on leave from Istanbul University and working at this Department as

a Carnegie Institution Fellow. Tritium-labeled thymidine was used in some of these experiments, and we are indebted to Dr. Philip Woods, of Brookhaven National Laboratory, for a supply of this nucleoside and for his counsel and guidance in obtaining a series of radioautographs, which were prepared in his laboratory.

Throughout the year we have had the services of Florence Powell on a part-time basis; Lois Glass, Grace Bert, and Grace Fochtman assisted during the summer months. The devoted attention of these workers to operational details has been of great service in furthering our research efforts.

### *Patterns of Organization of Cellular Materials*

*Enzymatic degradation of the chromosome.* Methods of dissecting the chromosomes with enzymes, originally developed for localization of constituent materials and identification of their patterns of organization at the level of resolution of the ordinary light microscope, have now been extended to the realm of fine detail disclosed by the electron microscope. As was reported in Year Book 55, the buffered osmium tetroxide used so extensively in preserving material for electron microscopy makes tissues more resistant to enzymatic hydrolysis than the acetic-alcohol fixative employed in our earlier cytochemical studies. To compensate for this difficulty, two modifications in customary procedures have been made. One involves the use of buffered formalin, as material fixed with this solution is more readily degraded by enzymes than osmium-fixed material but is sufficiently well preserved to permit a valid comparison of results of the two techniques. The other modification involves the use of solutions containing much higher concentrations of enzyme than are customary, with replacement at frequent intervals—a procedure made possible by our earlier efforts in extracting, crystallizing, and accumulating stock piles of the essential nucleases and proteases.



The second method was followed by Gay and Fuscaldo last year to induce effective enzymatic hydrolysis with deoxyribonuclease. Digesting intact *Drosophila* salivary glands fixed in buffered osmium or Dalton's fixative (a chrom-osmic solution), they obtained preparations that were completely Feulgen negative when examined with the light microscope. Reproducibility of this result in successive experiments was not consistent, however; that is, a given quantity of enzyme, which caused complete Feulgen reduction on one occasion, seemed ineffective in hydrolyzing deoxyribonucleic acid (DNA) in other trials. This variability was more pronounced with material fixed in buffered osmium than with Dalton-fixed tissues.

Gay and Yoshida have now investigated this situation and determined that some of the variability is due to differences in the samples of crystalline deoxyribonuclease used. Among numerous preparations, all having high activity and little or no proteolytic contamination, and all prepared in exactly the same manner by McDonald, some are much more readily soluble than others in dilute solutions of magnesium sulfate, and these are the most effective for cytochemical enzymatic hydrolysis.

Gay's original success in inducing complete digestion of Feulgen-stainable chromosomal materials with deoxyribonuclease was obtained with Dalton-fixed salivary-gland cells. Electron micrographs of this material (pl. 1, A and B) showed a considerable reduction in density of the chromosome in both the band and interband regions, although some very dense material in the form of thin lines remained in the bands. With buffered osmium-fixed salivary glands the result was somewhat different; there was no striking over-all reduction in density of the chromosomes, but only a moderate decrease within the bands and little, if any, in the interband regions, although the preparations were completely Feulgen negative (pl. 1, C and D).

It had been shown by J. Barton that in

isolated nuclei not all the DNA is hydrolyzed by the nuclease, but that some is degraded by the acid hydrolysis of the Feulgen procedure and the remainder by subsequent treatment with deoxyribonuclease. Yoshida has now applied this sequence of multiple treatments to the resistant buffered osmium-fixed material. Electron micrographs show that, through successive treatments with nuclease, hydrochloric acid, and then nuclease again, more and more electron-scattering material is lost from the bands until their density is reduced to that of the interband regions. The latter do not show any appreciable change in density, by comparison with nucleoplasm, in the course of the treatments (pl. 1, E and F). These findings are at variance with those obtained with Dalton-fixed material, since one would expect that complete hydrolysis of DNA by nuclease and acid would cause the buffered osmium-fixed chromosomes to look like the chrom-osmic-fixed material.

Additional observations along these lines have been made by De, who used formalin-fixed meiotic metaphase chromosomes of *Tradescantia* as the substrate for deoxyribonuclease digestion. In electron micrographs (pl. 1, G and H), these chromosomes showed almost a complete over-all loss of density, somewhat analogous to that observed after deoxyribonuclease treatment of Dalton-fixed salivary-gland chromosomes.

Additional cytochemical and spectrophotometric determinations must now be made to discover whether some fixatives preserve the nucleoproteins in such a manner that deoxyribonuclease digestion allows non-DNA material to leach from the chromosomes. Our preliminary staining tests indicate that a considerable amount of fast green-stained material remains on the chromosomes after complete Feulgen reduction, but it is recognized that in the realm of fine detail this may not be a sufficiently sensitive index of selective loss of DNA along with retention of protein.

Since these methods do not yet enable

## PLATES



### Plate 1

Electron micrographs of ultrathin sections of tissues imbedded in *n*-butyl methacrylate. Figures A to F are sections of salivary-gland cells of mid-third instar of *Drosophila melanogaster*. Figures G and H are sections of first meiotic metaphase chromosomes of *Tradescantia reflexa*. All enzymatic treatments were administered after fixation but before imbedding. Magnifications: A-F,  $\times 5800$ ; G, H,  $\times 4800$ .

A, B. Fixed in Dalton's fluid, pH 6.5. A, not treated with deoxyribonuclease. B, digested in deoxyribonuclease (0.1 mg/ml crystalline deoxyribonuclease in 0.003 M  $\text{MgSO}_4$  soln.) for 4 hours at 37° C.

C-F. Fixed in 1.25 per cent osmium tetroxide solution, adjusted to pH 6.5 with M/15 phosphate buffer, and made isotonic with NaCl. C, not treated with deoxyribonuclease. D, digested in deoxyribonuclease (5 mg/ml crystalline

deoxyribonuclease in 0.003 M  $\text{MgSO}_4$  soln.) for 7 hours at 37° C. E, digested in deoxyribonuclease as in D (above) and then treated with 0.2 N HCl solution for ½ hour at room temperature. F, digested and treated as in E (above), and digested again in deoxyribonuclease solution for 10 hours at 37° C.

G, H. Fixed in buffered formalin pH 6.5, and osmicated in 1.25 per cent buffered osmium tetroxide pH 6.5 after enzymatic or control treatment. G, not treated with deoxyribonuclease, i.e., treated with 0.003 M  $\text{MgSO}_4$  solution for 4 hours at 37° C, and osmicated. The dense body at right of cell is a metaphase chromosome. H, digested in deoxyribonuclease (5 mg/ml crystalline deoxyribonuclease in 0.003 M  $\text{MgSO}_4$  soln.) for 4 hours at 37° C, and osmicated. This cell contains two digested chromosomes.



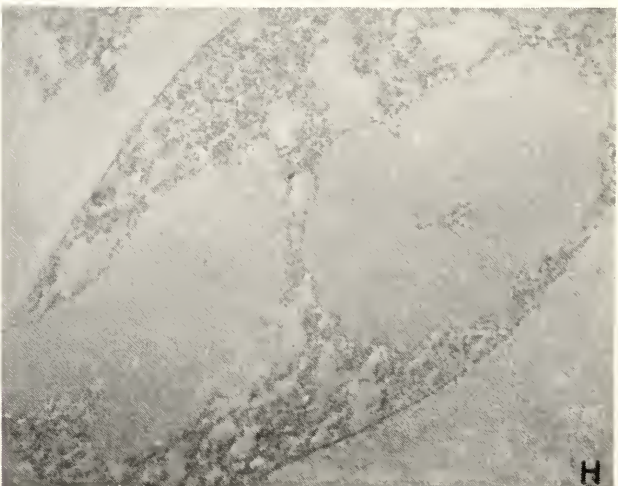
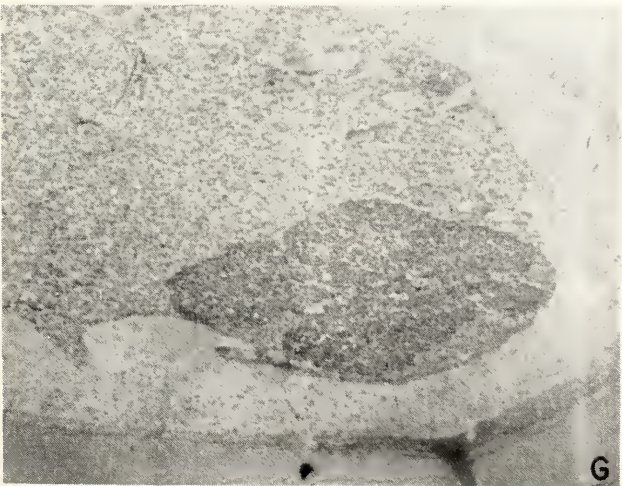
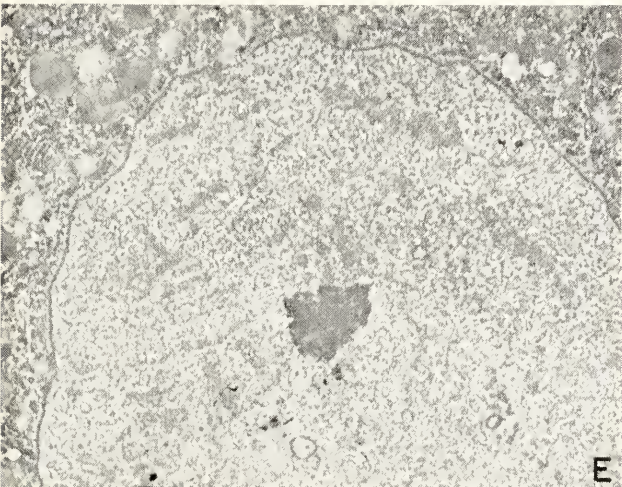
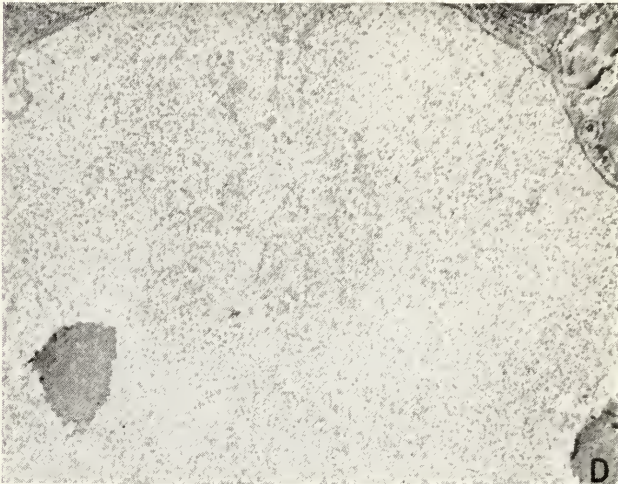
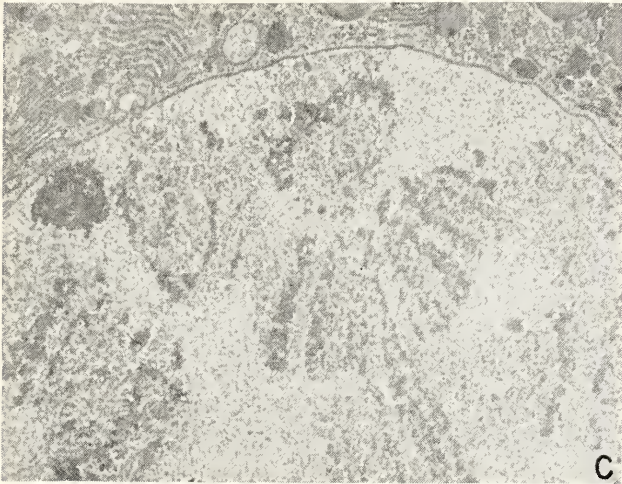
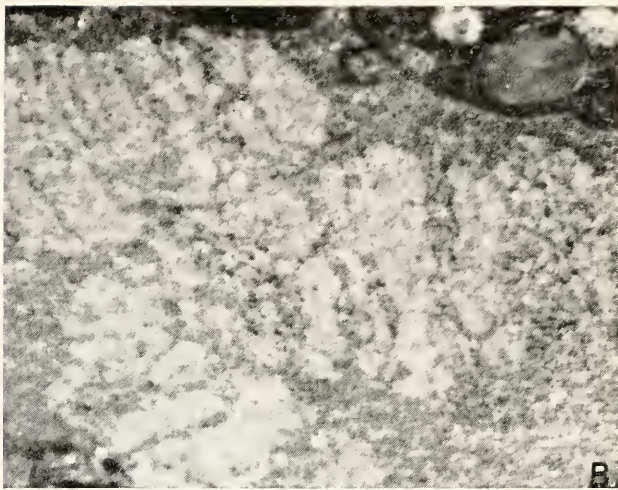
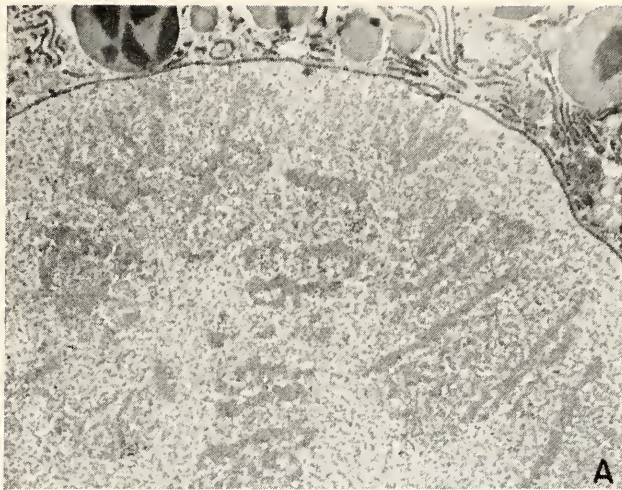




Plate 2

Electron micrographs of ultrathin sections of *Drosophila melanogaster* pupal testis fixed in 1.25 per cent osmium tetroxide solution (adjusted to pH 6.5 with M/15 phosphate buffer and made isotonic with NaCl) and imbedded in *n*-butyl methacrylate. Magnification of all figures,  $\times 7250$ .

A. Longitudinal section of secondary spermatocyte in telophase. An accumulation of ergastoplasmic lamellae surrounds the nuclear region.

B. The nucleus in late telophase of a secondary spermatocyte or in a prespermatid stage. There is an accumulation of ergastoplasmic lamellae around the nucleus. The anterior portion of this cell (seen in the upper part of the figure) is very strongly basophilic with azure B

stain, whereas the posterior part, which has many large mitochondria, is not.

C. A spermatid nucleus. The acrosome is seen at one side of the nucleus, and on the opposite side the nuclear membrane appears thickened.

D. A nebenkern. In the spermatid the nebenkern is formed from the mitochondria, which first migrate to a region adjacent to the nucleus, opposite the acrosome, and then fuse to form concentric rings. The mass enlarges and then divides into two parts.

E. A spermatid nucleus and a nebenkern. The nuclear membrane appears thicker on the nebenkern side of the spermatid nucleus. A part of the acrosome is seen near the opposite side of the nucleus.



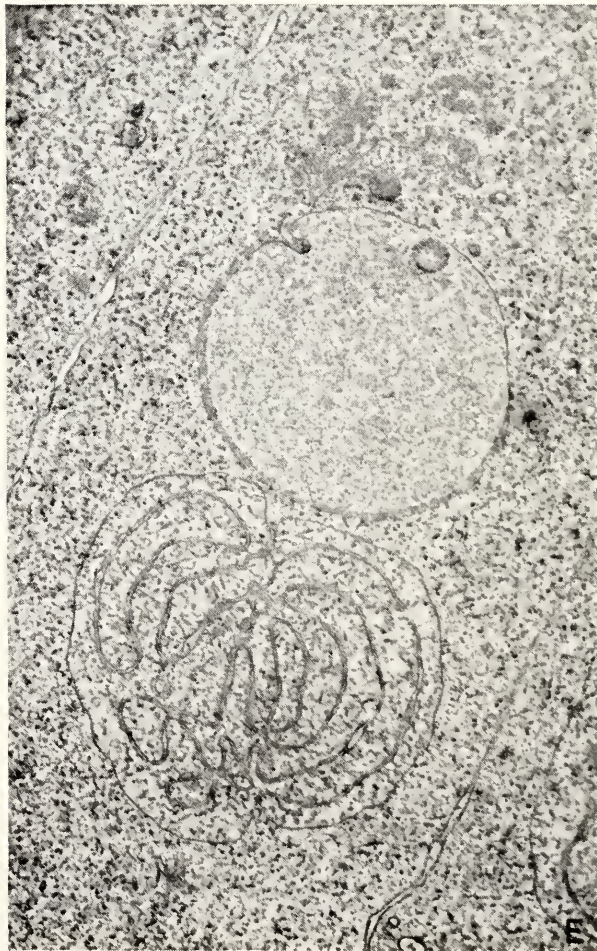
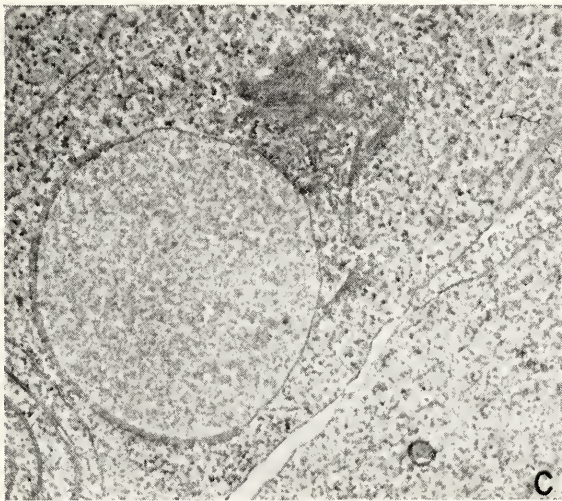




Plate 3

Electron micrographs of ultrathin sections of *Drosophila melanogaster* pupal testis fixed in 1.25 per cent osmium tetroxide solution (adjusted to pH 6.5 with M/15 phosphate buffer and made isotonic with NaCl) and imbedded in *n*-butyl methacrylate. Magnification of all figures,  $\times 7250$ .

A. Longitudinal sections of two spermatids. At the left, cross section of a nucleus, nebenkern, and intercellular bridge (arrow). The nuclear membrane near the nebenkern is thickened by accumulation of parallel double-layered lamellae. At the right, a spermatid with nebenkern and acrosome.

B. Later spermatid. The nucleus contains a dense spherical nucleolus, and the nuclear membrane on the side nearest the nebenkern is thickened. The nebenkern at this stage begins to

elongate, and its internal contents become less dense, losing some of their earlier structural symmetry.

C, D. Longitudinal sections of young spermatozoa. Along one side of long axis of nucleus, note dense material (arrow) adjacent to the membrane. This is very strongly basophilic with azure B stain. On the nuclear side of the membrane some chromatin strands are denser than others and stain with a positive Feulgen reaction.

E. Cross section of young spermatozoa. Note (arrow) ringlike structures, which may be tangential sections of the nuclear membrane.

F. Cross section of spermatogonia. Note (arrow) intercellular bridge, with thickened dense cellular borders at its narrowest part.



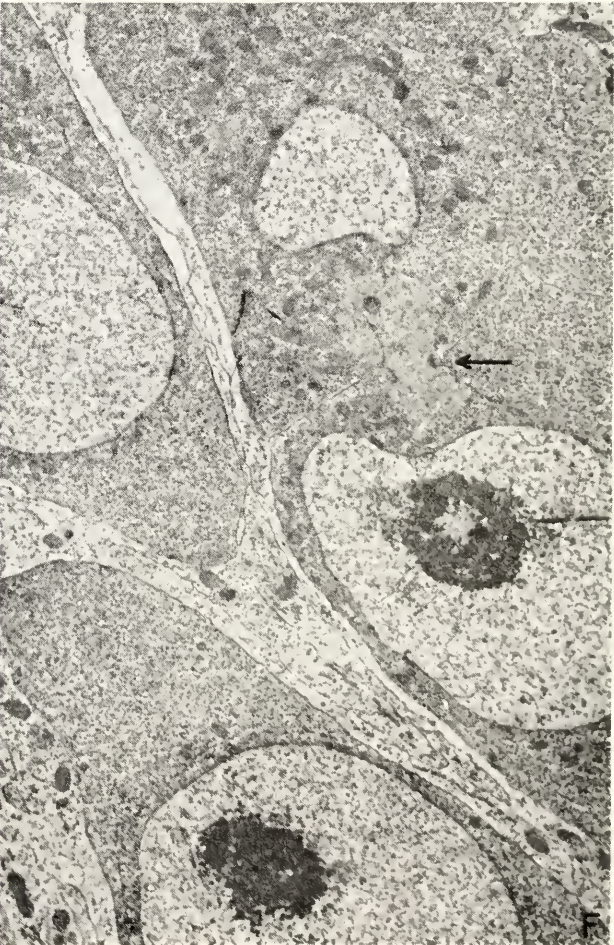
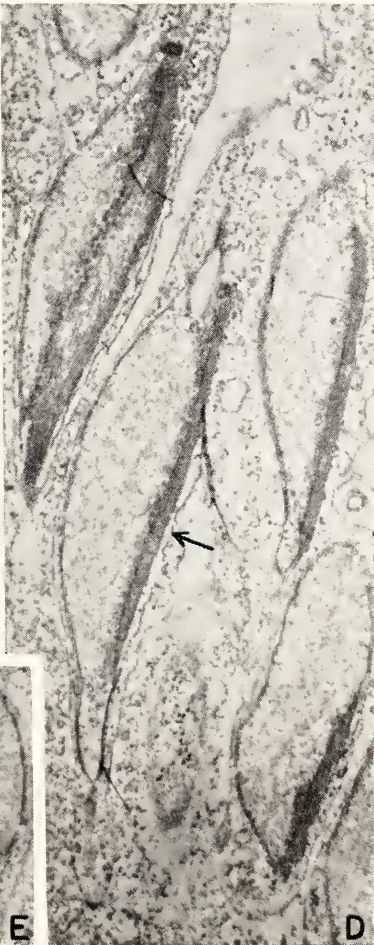
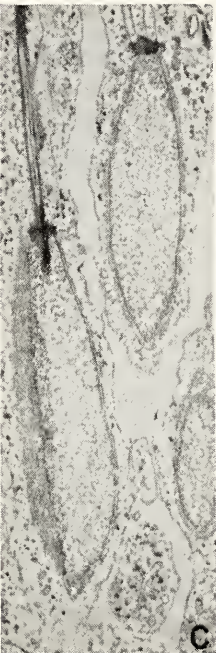




Plate 4

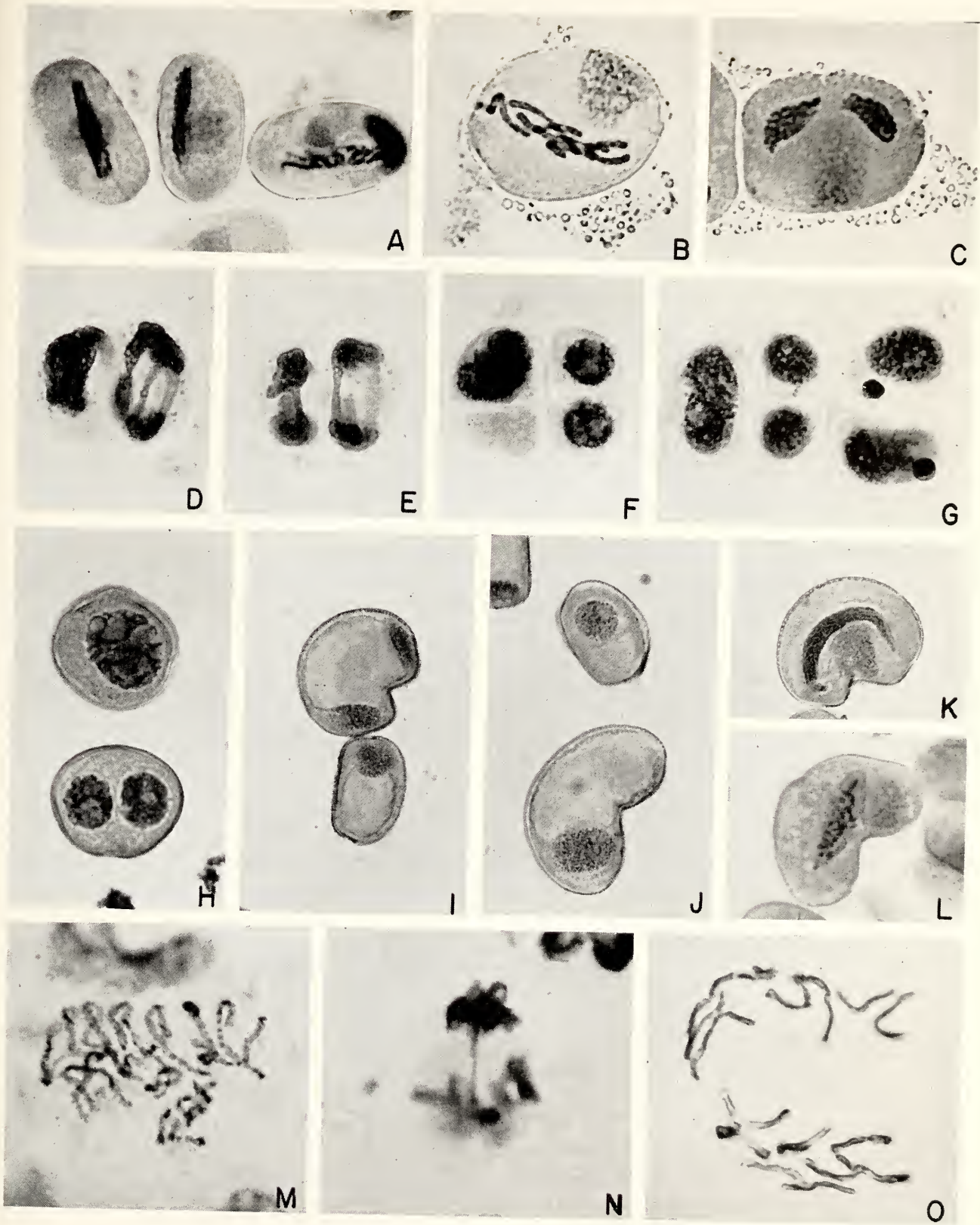
Modification of mitotic processes in plants: A-L, in the spiderwort, *Tradescantia*, during production of the microspores (pollen grains) and in postmeiotic divisions; M-O, in root-tip mitoses of the onion, *Allium*.

A, pollen grain with weakly staining vegetative nucleus and densely staining generative nucleus. B, initiation of division of the generative nucleus within the ungerminated pollen grain after exposure to high temperature. C, completion of division of the generative nucleus.

D-L, action of ribonuclease in production of diploid pollen grains. D-G, induced "stickiness" interferes with normal progress of second meiotic division, so that some cells receive two sets of chromosomes, others none (F). H, separating microspores may have diploid complex in one

nucleus or in two nuclei. I-L, developing pollen grains. Lobulation of the larger grains results from failure of completion of wall formation. I, binucleate diploid pollen grain above, normal haploid below. J, normal haploid above, uninucleate diploid below. K, diploid vegetative and generative nuclei, produced from the type shown in J. L, division of generative nucleus, initiated by temperature treatment.

M-O. Effect of gibberellic acid in onion root-tip cells. M, 100 ppm, 6 hours; prometaphase chromosomes, tightly coiled, simulating banded structures. N, 10 ppm, 48 hours; anaphase bridge caused by "stickiness." O, 1 ppm, 120 hours; separation of diploid complex into two groups by disturbance of prophase distribution and failure of congression on spindle.







us to localize the DNA with respect to the fine structure of the chromosome, we can only propose tentative interpretations. The results of experiments involving digestion of formalin-fixed or chrom-osmic-fixed materials suggest that the DNA-protein fibers run the length of the chromosome and are its integral structural component. The effects of deoxyribonuclease treatment of buffered osmium-fixed material, on the other hand, indicate that the main structural fibers of the salivary-gland chromosomes are immune to nuclease or acid hydrolysis and that the DNA is attached to the main chromosomal axis as side chains. This is the interpretation we presented many years ago (see Year Book 48) on the basis of light-microscope observations of the effects of nucleases and proteases on acetic-alcohol-fixed plant and animal tissues. The two alternative interpretations might be reconciled on the assumption that the DNA is attached laterally, but that the confluence or propinquity of adjoining side chains is emphasized more with some kinds of fixation treatment than with others, so as to give the appearance of linear continuity of both the protein and the nucleic acid moieties of the structural nucleoproteins.

*Additional notes on structure of the salivary-gland chromosome.* According to the most widely accepted interpretation, the giant chromosomes of the Diptera represent bundles of intertwined strands or chromonemata, each subdivided linearly into chromomeric and interchromomeric regions, which by their close apposition produce the characteristic and specific pattern of banding that is so strikingly displayed in the salivary-gland chromosomes of *Drosophila melanogaster*. Differences of opinion exist, however, with respect to the precise number of component strands at a given stage of development and the method of origin of the banded chromosome from its coiled mitotic-type progenitor. Most cytologists are inclined to the view that the great length of the salivary-gland chromosomes depends, at least in

part, on the uncoiling of the chromonemata that were present when the definitive salivary-gland cells were set aside in the early stages of embryonic development, and that the appearance of banding arises from the juxtaposition of homologous chromomeric and interchromomeric regions of these elongate chromonemata. On the other hand, a few workers have consistently maintained that the salivary-gland chromosomes are visibly coiled during the early stages of larval growth, and that the subsequent appearance of banding results from disruption of the coils into a series of disconnected segments. If this interpretation is adopted, the widely accepted concept of a multistranded (or polytene) pattern of organization of the giant chromosome is suspect.

In an effort to resolve these conflicting interpretations, an analysis of the development of the salivary-gland chromosomes of *D. melanogaster* was undertaken during the past six months by Sengün, working in collaboration with Kaufmann. The experimental approaches included a repetition of earlier work by A. Marshak and B. M. Slizynski, to determine whether "partial rearrangements" would occur in third-instar salivary-gland chromosomes of individuals exposed to X-ray treatment during embryonic or early larval stages, and a new approach determining the pattern of distribution of radioactive tritium ( $H^3$ ) in third-instar salivary-gland chromosomes of individuals that had ingested or absorbed tritium-tagged thymidine during earlier stages of development. The latter experiments were carried out at Brookhaven National Laboratory in collaboration with Dr. Philip Woods.

The X-ray experiments were formulated on the premise that, if a chromosome was multistranded at the time of treatment, inter- or intrachromosomal rearrangements involving fractions of the width rather than the entire breadth of a homologue might subsequently be detected. On the assumption that each mitotic-type progenitor is at least double at the time it fuses



with its homologue to form the paired unit characteristic of the salivary-gland cell—a point of view that had been substantiated in our earlier cytogenetic studies—X-ray-induced rearrangements involving one-fourth of the breadth of the “bivalent” might be anticipated even if the chromosomes developed without further replication of chromonemata. Rearrangements involving smaller fractions of the width of the chromosome would be attributable, by the same criterion of selective breakage, to the existence at the time of treatment of a larger number of subsidiary units. On theoretical grounds this could be occasioned by a multistranded state of the mitotic-type progenitor chromosome or, alternatively, by the initiation of endomitotic replication at a very early stage of development of the salivary-gland chromosome.

To test these alternatives, embryos and larvae, raised at 25° C, were irradiated with 500 r or 3000 r of X rays, 4–6, 10–12, 16–18, or 56–58 hours after oviposition. Few rearrangements were detected in the 500-r experiments, but “fractional rearrangements” were obtained after exposure of the 4–6, 10–12, and 16–18 hour groups to 3000 r. No rearrangements were observed in the 56–58 hour group. For the most part, the rearrangements were intrachromosomal (small inversions, duplications, deficiencies) and ranged in width from one-half to one-sixteenth (or perhaps one-thirty-second) of the diameter of the chromosome, indicating that in some cases an entire homologue, in others as little as one-eighth (or perhaps one-sixteenth), had been broken selectively by the X rays. When large fractions of the diameter of the homologue were involved in rearrangement, the linear pattern of banding of the displaced segment was microscopically discernible; but when smaller fractions were involved the details of banding (if bands were indeed present) were not clearly defined, so that it was not always possible to distinguish between an induced rearrangement and a nonspecific pairing.

In interpreting these results, the produc-

tion of fractionals within the 4–6 hour group seems to be of particular significance, inasmuch as the definitive salivary-gland cells are not set aside until 9–12 hours after oviposition in cultures raised at 25° C. It would thus appear that the fractionals produced by irradiation of the 4–6 and perhaps the 10–12 hour groups can be explained most readily by assuming a multistranded condition of the mitotic-type chromosomes from which the salivary-gland-type chromosomes are developed. This assumption is consonant with current interpretations derived from studies with the electron microscope, which suggest that chromosomes consist of a large number of chromonemata at all stages of mitosis (Year Book 55); but it does raise the question why X rays should produce half-, quarter-, and eighth-chromatid breaks in these cells, when only half-chromosome (or chromatid) breaks were detected in our earlier studies of mosaics in salivary-gland cells of individuals arising from eggs fertilized by irradiated spermatozoa. Perhaps the answer lies in the fact that the rearrangements induced in salivary-gland chromosomes are not subjected to mitotic selection after the time of irradiation, whereas the rearrangements induced in spermatozoa must pass through the series of cleavage mitoses initiated by the zygote.

If this is so, the factor determining survival or elimination may reside in the integrative mechanism that enables a group of experimentally separable chromonemata to function in unison as a chromatid (see Year Book 55). We have stated elsewhere our reasons for assuming that RNA is involved in this type of control (Year Books 54, 55) although definitive experimental evidence is still lacking. Assuming the existence of such a mechanism, any structural rearrangement that interfered with its successful operation by modifying the patterns of distribution and attachment of the materials involved might impair the capacity of a chromatid to function as a unit, and lead eventually to its elimination. The possibilities of such a

hypothesis for explaining many of the perplexities of radiation biology are apparent, but profitable speculation must be delayed until more precise information is in hand.

The experiments with  $H^3$ -tagged thymidine were based on the premise, derived from the work of J. H. Taylor, P. S. Woods, and W. L. Hughes, that this nucleoside would be built into the DNA that was synthesized during the period of its availability within the cell. Since the salivary-gland chromosomes assumedly attain higher and higher degrees of polyteny as they continue to grow (as suggested, for example, by quantitative spectrophotometric studies of their DNA content), it should be possible to follow the pattern of incorporation of tagged thymidine and to determine the course of its distribution among the chromonemata arising after the time of its utilization in DNA synthesis. In an effort to test this hypothesis, larvae that had just emerged from the egg, and others 6, 12, and 24 hours old, were either placed on food containing  $H^3$ -thymidine or immersed in a solution containing the nucleoside for a period of 6, 12, or 49 hours, and were then transferred to normal food (without thymidine). Still others were kept throughout larval life on food containing the radioactive material. Squash preparations, stained by the Feulgen method, were made of the salivary-gland chromosomes of the treated individuals at the time the third-instar larvae were starting to pupate. These preparations were used in radioautographic studies.

The few preparations that have been examined at the time of writing this report show that thymidine can be incorporated in the chromosomes. Its distribution appears to be restricted to the banded regions, since the radioautographs reveal practically no disintegration of tritium in the intervals between bands. The most spectacular radioautographs are those obtained from chromosomes of larvae that were fed labeled food throughout the period of their development. The bands are clearly de-

fined, often as a row of dots traversing the width of the chromosome (representing the effects of disintegration on the overlying film). In preparations from larvae that were transferred to normal food after two days or less on the thymidine-containing food, the results of disintegration are much more localized, at times appearing as single dots within given bands. In a few chromosomes the pattern made by the disintegrations in successive bands along the chromosome suggests that a continuous strand may be involved.

The findings thus appear to support the generally accepted theory that the salivary-gland chromosome grows in diameter by repeated replication of the original chromonemata. This, it must be emphasized, is a tentative conclusion as far as the results obtained with  $H^3$ -thymidine are concerned; a more precise answer should be obtainable within the next few months from preparations that are still being permitted to register their radioactivity on undeveloped film.

Finally, with respect to the view that the banded appearance of the salivary-gland chromosome results from disruption of a single coiled chromonema, it may be noted that according to our observations the salivary-gland chromosomes of *D. melanogaster* during second instar are helically disposed but each shows a series of clearly defined bands throughout its length. Admittedly, the pattern is not so strikingly evident as that discerned in third-instar chromosomes, but a sufficiently large number of the more prominent bands is apparent to permit identification of the individual chromosomes. It therefore appears that any conjectured disruption of the helical pattern to form bands would involve subsidiary coils rather than the primary helices of these chromosomes. Thus, a study of the development of the salivary-gland chromosomes of *D. melanogaster* lends little support to the interpretation, which rests primarily on observations of chromosomes of the related genus, *Chironomus*, that the appearance of banding



results from disruption of the standard coils into a series of disconnected segments.

*Fine structure of the nuclear membrane of plant cells.* Electron-microscope studies of various types of animal cells, including those of salivary-gland cells of *Drosophila* reported by Gay in Year Book 54, have shown that the nuclear membrane is a double-layered fenestrated envelope with raised annuli surrounding "porelike" areas. A comparable structure of the nuclear membrane of the staminate-hair cells of the spiderwort, *Tradescantia*, was reported in Year Book 55 by Kaufmann and De. These studies have now been extended to include pollen mother cells of this plant.

A nuclear membrane is observed in prophase stages of staminate-hair cells and at diplotene stage, diakinesis, and first-division telophase of the pollen mother cells. At diakinesis the membrane sometimes looks interrupted, a possible prelude to its dissolution as the cell approaches metaphase. No such interruption is seen at first-division telophase.

In cross-sectional view, the membrane appears as a double layer, with two parallel dark lines separated by a non-electron-scattering zone. This zone averages about 140 Å in width. The total thickness of the membrane in cross sections is about 310 Å. In tangential view the membrane shows a reticulate structural pattern. Highly electron-scattering materials form a ring around the less electron-dense "pores," which are approximately circular in outline. The outside diameter of the pores is about 650 Å, the inside diameter about 380 Å, the distance from center to center about 1000 Å. These dimensions are approximately equivalent in somatic and meiotic cells.

The observations recorded here are in general agreement with those made in studies of animal cells. It might seem superfluous to stress the uniformity of structural pattern, except that some students of plant cells have concluded that the nucleus is not delimited by a well defined structural membrane. The presence

of such a membrane in *Tradescantia*, with patterns of "sculpturing" similar to those seen in animal cells, suggests that the details of organization of the nuclear membrane are essentially the same in plants and animals.

### *Nuclear-Cytoplasmic Interrelations*

*Nucleocytoplasmic interrelations in Drosophila.* In Year Book 55, Gay reported that blebbing of the nuclear membrane in *Drosophila* salivary-gland cells occurred at a specific stage of development, namely, the middle of the third instar of larval life, and seemed to be correlated with the assumption by the cell of a new functional activity. To obtain further evidence related to this hypothesis, she has attempted during the past year to treat living salivary-gland cells with chemicals that might enhance or inhibit blebbing of the nuclear membrane and the associated chromosomal "secretion." The substances used included ribonuclease, pilocarpine, and chloramphenicol, all in aqueous solution. Several pilot experiments were undertaken, involving variations in the method and time of administration of each of these agents. Of the three methods of treatment tested—feeding, immersion, and injection of the dissolved substances into the body cavity—the last two seemed to cause less cellular change in control larvae. Treatments were carried out either at the time of the molt that marks the transition from second to third instar or shortly before mid-third instar.

The results of these treatments were determined by examining electron micrographs of glands that had been fixed in buffered osmium tetroxide. Chloramphenicol gave no evidence of affecting nuclear-membrane blebbing, although other cellular changes indicated that the chemical had entered the cell. This negative result suggests that in salivary-gland tissue chloramphenicol does not affect protein synthesis (although it is capable of inhibiting that synthesis in some microorganisms),

but it also implies that blebbing of the nuclear membrane is probably not due to an infective agent, either bacterial or viral.

Ribonuclease caused a questionable increase in frequency of nuclear-membrane blebs; the point will be reinvestigated with a series of concentrations of the enzyme, since recent reports of ribonuclease treatment of living cells in *Acetabularia* (an alga) by H. Stich and W. Plaut indicate a profound effect on nuclear-initiated protein synthesis.

The results of pilocarpine treatment, however, offered some positive evidence of an increase in number of outpocketings in the mid-third-instar cell, as judged by a subjective appraisal of electron micrographs. These results will be checked in the immediate future by a sampling technique recently devised for estimating the number of blebs per nucleus. Pilocarpine, when administered to either vertebrate or invertebrate animals, stimulates the formation of secretions by glandular cells. This effect is manifested in the *Drosophila* mid-third-instar salivary-gland cell by premature discharge into the lumen of the secretion—stained by the periodic acid-Schiff (PAS) technique—which is formed from the PAS-positive cytoplasmic secretion granules, as noted in Year Book 55. We have not yet determined the behavior of the cell, after depletion of the secretion granules, by observation of cells of treated larvae that have been allowed to recover. It is thought that in insects pilocarpine affects glandular secretion by acting on the parasympathetic nervous system, which in turn stimulates release of the secretion from the cell. It must therefore be supposed that the pilocarpine effect upon the nucleus in *Drosophila* salivary-gland cells is a demand feedback mechanism, induced by depletion of the PAS-positive secretion granules from the cytoplasm. Whether the increased nuclear-membrane blebbing that occurs at this time can effectively reinitiate the process to replenish the store of secre-

tion granules remains to be determined. If release of secretion into the lumen of the gland triggers an over-all hormonal or parasympathetic-nervous-system reaction which leads to premature pupation, the nuclear reaction may be suppressed. In any event, the observations show that pilocarpine is effective in modifying the phenomenon of nuclear blebbing in salivary-gland cells of *Drosophila* third-instar larvae.

An attempt has been made to modify nuclear-membrane blebbing by a cytogenetic method. It was suggested in Year Book 55 that intercalary heterochromatin seemed to be involved in blebbing, and that heterochromatin per se might be responsible for the phenomenon. To test this hypothesis, Gay made use of a strain of Oregon-R (developed by E. Novitski and D. Lindsley) containing an extra heterochromatic Y chromosome attached to the X chromosome. By making appropriate crosses, females with an extra Y chromosome and males with no Y can be obtained. A comparison is therefore possible between XX and XXY females, and between XY and XO males, to determine the effect of the extra quantity of heterochromatin on cellular phenomena. Elizabeth Patterson had previously reported that no appreciable difference in the cytoplasmic RNA content of salivary-gland cells of these same stocks could be attributed to the presence of an extra Y chromosome. It was thought, however, that observation of changes due to a possible increase in cytoplasmic lamellae resulting from nuclear-membrane blebbing might be a more sensitive method than biochemical techniques for detecting such differences. Accordingly, the number of blebs per nucleus was estimated in electron micrographs of salivary-gland cells from larvae of comparable ages. No appreciable difference in number of outpocketings in the salivary-gland cells was found among larvae of the four different types of chromosomal constitution. Although this negative finding demonstrates that quantitative differ-



ences in Y-chromosome heterochromatin have no effect upon nuclear-membrane blebbing in salivary-gland cells, it does not preclude the implication of intercalary heterochromatin in this phenomenon. Experimental procedures to test that hypothesis, however, are not apparent at the moment.

*Nuclear and cytoplasmic changes during spermiogenesis in Drosophila.* In Year Book 55, a brief report was made of the initiation of a study of spermiogenesis in *D. melanogaster* during the summer of 1955. Yoshida, who joined the group in October 1956, has now continued that study in collaboration with Gay. The basic problem concerns the changes in fine structure of the chromosomes during the course of meiosis leading to formation of the mature spermatozoon—that is, the way in which the genetic material of the male parent is “packaged” for transfer to the egg. (There is general agreement that in some types of spermatozoa, at least, the essential chemical components are DNA and protamine.) Of equal importance are the nuclear and cytoplasmic changes undergone during differentiation of the cell into a very highly specialized functional unit.

Although on first inspection the sperm nucleus seems to contain little beyond fine coiled fibers, whose identity is apparently lost as the spermatozoon matures, we have made numerous observations of differentiated osmiophilic structures in the resting nucleus after the second meiotic division and in the spermatid and young spermatozoon. Some of these structures appear to be nucleoli, and others highly condensed chromatin strands. We have not sufficient data yet, either electron microscopic or cytochemical, to determine the origin or fate of these structures, and can therefore only describe our fragmentary observations, in the expectation that they can soon be fitted into their proper places in the sequence of orderly nuclear changes occurring during spermiogenesis.

In the young spermatozoon, just as the

nucleus begins to elongate, there appear localized accumulations of dense chromatin strands on the side of the nucleus where the nuclear membrane and the adjacent cytoplasm are highly differentiated (see pl. 3, C, D, and E). These strands, which appear Feulgen positive when examined in stained preparations, do not seem to be aggregated to form chromosomes. The rest of the nuclear material does not look very different from the fine coiled fibers seen in the spermatid nucleus. Whether such localization and apparent thickening of chromatin fibers at this stage reflect chromosomal activity, or represent differential changes necessary for packing the chromatin filaments into the mature spermatozoon, remains to be determined.

In addition to these chromatin changes, we have observed in the spermatid a small dense spherical nucleolus, which subsequently enlarges but remains round and compact (see pl. 2, E, and pl. 3, B). This nucleolus is very different in structure from the large lobular nucleolus of the primary spermatocyte; but both are highly basophilic when stained with azure, indicating a similarity in chemical constitution. We are now trying to determine whether, during the transition to the mature spermatozoon, the nucleolus functions in the aggregation of chromatin strands to one side of the nucleus in a manner suggestive of the nucleolar activity responsible for establishment of the “bouquet” stage in early meiotic prophase of many organisms.

A more complete series of cellular changes has been observed with respect to modifications in nuclear size and contours, and, in particular, modifications of the nuclear membrane during spermiogenesis. The findings seem to have a bearing on our earlier studies of nucleocytoplasmic interrelations in differentiation, and therefore will be briefly reported here.

In the transition from secondary spermatocyte to spermatid, many layers of cytoplasmic lamellae accumulate around the

nucleus. Examination of azure B-stained preparations under the light microscope reveals that the nucleus at this stage is encircled by a very thick layer of dense basophilic material, suggesting that the lamellae contain ribonucleic acid. Our attempts to determine the origin of these basophilic double-layered membranes have necessitated a study of the second meiotic division. The preliminary electron-micrograph and light-microscope observations suggest that a thick envelope of basophilic lamellae surrounds the interkinetic nucleus and persists throughout the second meiotic division (pl. 2, A and B), clearly delimiting the spindle area and the reconstructing nuclei from the rest of the cytoplasm. Our pictures of primary spermatocytes do not show any double-layered membranes in the cytoplasm or near the nucleus; and so at present we assume that the basophilic lamellae originate during interkinesis or at the end of the first meiotic division, although the mechanism of their formation has not been detected.

Observations indicating the fate of these lamellae in the spermatid and during the transition to the spermatozoon are more complete. After the second meiotic division, the mitochondria, which were completely dispersed throughout the cytoplasm, enlarge and accumulate near one end of the nucleus. The lamellar, granular, Golgi-like material, which presumably will produce the acrosome, is at the opposite end of the cell (pl. 2, B). As the spermatid develops, the nucleus becomes smaller and almost precisely spherical; the lamellae, which at first surrounded the nucleus as a mantle of nearly uniform thickness (pl. 2, A and B), become arranged along the side toward which the mitochondria have migrated to form the nebenkern (pl. 2, C and E). The acrosome develops from Golgi-lamellar material on the opposite side of the nucleus (pl. 2, D and E). Cytochemical tests have shown that the region of the nuclear membrane which appears thicker is highly basophilic when

stained with azure B and is negative to the PAS test. The latter method was applied to determine whether this material had any relation to the acrosomal cap, which is polysaccharide in nature.

In passing, it may be interesting to note the process of nebenkern formation shown in plate 2, C and E, and plate 3, A and B, since it affords a spectacular demonstration, at the ultrafine level of organization, of the kinds of changes that take place in differentiation. Formation of the nebenkern occurs by aggregation of the mitochondria into a single spherical mass, and fusion of their outer membranes to produce concentric rings. The internal structure of the mitochondria seemingly degenerates (pl. 2, C and E). The formed nebenkern enlarges, and then divides into two hemispheres, one on either side of the point of origin of the tail (pl. 3, A and B). At a later stage, the nebenkern mass elongates, losing its internal structure, to make the paired ribbon-shaped mitochondrial sheaths on either side of the axial filament.

In the young spermatozoon, the nucleus grows smaller and then elongates to become first oval and later greatly attenuated, as it appears in the mature sperm. The nuclear membrane is clearly double, but along one side there is an accumulation of very thick and dense material (pl. 3, C, D, and E), which appears to be related to the lamellar accumulation observed in the spermatid. It is highly basophilic and produces no color in the PAS test. As the spermatozoon nucleus elongates, however, this dense material seems to be fibrous rather than lamellar (pl. 3, C and D). It is hoped that future study will reveal the mechanism of the accumulation or production of lamellar sheets adjacent to the nuclear membrane, and will determine whether these double-layered lamellae contribute to the Golgi system and whether they are related to the fibrillar elements that thicken one side of the membrane in the spermatozoon.

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have observed accumulations of cytoplasmic lamellae near the nucleus in various types of oöcytes, and have concluded that they are related to the production of nutritive yolk material. In the development of the sperm, this accumulation, if analogous to that in the female germ cell, is not likely to be concerned with nutritional products, for the cytoplasm is largely pinched off and lost as maturation proceeds. In the male germ cell the accumulation of lamellae may be associated with production of the specialized sperm structures, such as the acrosome or tail. If our future analysis indicates such a correlation, our original hypothesis that the nuclear membrane is an active participant in differentiation will be sustained.

One additional observation regarding nucleocytoplasmic relations and differentiation concerns the presence of intercellular bridges, seen in spermatogonial and spermatid cells (pl. 3, A and F). These cytoplasmic connections do not seem to be transitory manifestations or artifacts, since a definite structural modification of the cell membrane is observed. At the "waist" of the intercellular connections, the cell membrane is thicker and denser (pl. 3, F). D. Fawcett has reported that in spermatogenesis of rodents these bridges occur between at least four adjacent cells. Whether more than one bridge occurs between any two cells has not been ascertained. It is suggested that the cytoplasmic connections may serve as a structural aid to synchronous development of a group of cells, a mechanism that in the *Drosophila* testis would be particularly useful, since no cyst walls, such as are common in some other insect testes, separate the cells in different stages of development.

*Development of the male gametophyte in Tradescantia.* A unique and challenging opportunity for the study of nucleocytoplasmic interrelations is offered in the development of the male gametophyte of angiosperms. Pollen grains or microspores of these plants are products of the two

meiotic divisions by which each microspore mother cell customarily forms a group of four cells. Adherent at the time of their formation, these four microspores soon separate to form independent pollen grains, each enclosing within its thick walls a haploid set of chromosomes capable of interacting with the cytoplasm in the production of the differentiated male gametophyte. Initially this process involves the formation within the cytoplasm of a large eccentrically placed vacuole, which displaces the nucleus to one end of the cell, whence it moves along the flattened inner wall to a characteristic median position. The nucleus then divides (the microspore mitosis), with the spindle lying across the shorter axis of the cell, to form the vegetative (or tube) and generative nuclei, which subsequently develop distinctive structural as well as functional properties. The vegetative nucleus, occupying a central position in the cell, enlarges to attain a more or less spherical shape, although it later appears star shaped or amoeboid because of the presence of marginal serrations or lobes, and stains weakly with acetic-orcein or the Feulgen reagent. The generative nucleus, on the contrary, lying close to the inner wall, elongates to form a crescentic body whose ends sometimes encircle the tube nucleus and whose chromosomes appear sharply defined and strongly Feulgen positive (pl. 4, A). In the spiderwort, *Tradescantia reflexa*, which we have used in our studies, dehiscence of the anthers occurs at this stage of development (under standard greenhouse conditions), and the pollen is shed. Upon reaching a receptive stigmatic surface, the pollen germinates and the tube develops. The generative nucleus, moving into the tube, divides to form the two male gamete nuclei; the vegetative nucleus may pass into the tube but does not divide at this stage.

Pollen grains of *Tradescantia* may be harvested from mature anthers and sprouted on agar blocks. Colchicine or acenaphthene added to the medium serves

to block the mitosis in the pollen tube at metaphase, so that the chromosomes can be counted easily (the haploid number is 6 in *T. reflexa*) and any experimentally induced changes readily detected. In a modification of this method developed by De, it was found that freshly harvested pollen grains would germinate to produce pollen tubes if placed on a chemically clean slide in a chamber whose atmosphere was saturated with water vapor. A few crystals of acenaphthene placed on the floor of the chamber provided a sufficiently high concentration of this reagent by vaporization to penetrate the pollen tubes and block mitoses at metaphase. In a further development of methods to facilitate study of the generative nucleus (in extension of earlier efforts of Karl Sax), Kaufmann found that certain culture conditions of the anthers, including precise control of temperature, would stimulate division of this nucleus within the pollen grain before anther dehiscence (pl. 4, B and C)—the normal sequence of events under ordinary culture conditions in some other genera of plants. This finding promises to simplify our problem of analysis of the fine structural changes involved in the development of the male gametophyte in *Tradescantia*, since both the microspore mitosis and the “pollen-tube” mitosis can now be followed within the “closed system” of the intact pollen grain.

A cytochemical study of the developing male gametophyte, made by Dr. Nebahat Yakar while she was a guest investigator at this laboratory in 1950 and 1951, indicated that marked changes occur in the amounts and patterns of distribution of nuclear and cytoplasmic nucleic acids and proteins during the sequence of events described above. Since she had observed particularly striking differences in concentration of ribonucleic acid (RNA)—as determined by the amount of ribonuclease-reducible basophilia—between vegetative and generative nuclei with their enveloping cytoplasms, experiments were recently

undertaken by Kaufmann to determine the effects on developmental processes of treatment of the living cells with the enzyme ribonuclease. To do this it was necessary to immerse the ends of freshly cut stamens in the solution to be tested, so that the enzyme could be moved by the transpiration stream into the anther. (Attempts to culture isolated microspores in fluid or semifluid media were not highly successful, although a considerable effort was devoted to the project and some progress was made in the development of adequate techniques.)

One of the most striking effects of the action of ribonuclease on microsporogenesis was the production of “sticky” and adherent chromosomes at anaphase and telophase of the second meiotic division (pl. 4, D and E). The resulting interchromosomal bridges resembled those seen at anaphase of somatic mitosis after the immersion of roots of onion in solutions of ribonuclease (see Year Book 52). The bridges interfered with the normal progress of cytokinesis—the shallow cleft in the outer wall of some of the developing pollen grains (shown in pl. 4, I-L) indicates that separation of sister cells had been initiated but not completed—and led thereby to the production of microspores with double the normal number of chromosomes, which were sometimes aggregated in a single diploid nucleus and sometimes present as two independent haploid nuclei (pl. 4, F-L). By maintaining conditions favoring division of the generative cell before dehiscence of the anther, it was possible to obtain types of pollen grains that had either one diploid vegetative nucleus and two diploid male nuclei or two haploid vegetative nuclei and four haploid gamete nuclei. Some diploid pollen grains occur in our greenhouse population of *Tradescantia*, particularly during periods of extreme climatic variability; but the production of binucleate pollen grains with high frequency under controlled experimental conditions offers a greater oppor-



tunity for analysis of the effects of nuclear competition and co-operation on the processes involved in formation of the gametophyte.

Our preliminary studies also suggest that ribonuclease has an effect on the development of the "bouquet" and the spiralization of the chromosomes during the prophases of the first meiotic division. Since the maneuvers of the chromosomes during these stages underlie the processes of crossing over, it seems important to pursue the available clues (reported in part in Year Book 55) to the possible role of RNA in effecting or modifying recombination in the chromosomes of higher plants and animals. Work along these lines is being continued.

### *Cytochemical Studies*

For several years we have devoted a considerable amount of effort to the validation and refinement of methods for identifying and localizing specific cellular components by enzymatic hydrolysis of sections of fixed tissue. In the course of these studies we have answered satisfactorily, on an experimental basis, most of the objections raised on theoretical and empirical grounds by other workers about the reliability of such methods. One of our own findings, however, has remained an enigmatic exception to the general rule: cytochrome c and other compounds containing the heme molecule may simulate ribonuclease in the ability to reduce cellular basophilia. Further efforts to explain this phenomenon are reviewed below. In the area of cellular chemistry we have also increased our efforts, as indicated below, to extract and crystallize a deoxyribonuclease that would serve more satisfactorily than preparations now available in analysis of the mechanism of intracellular synthesis of deoxyribonucleic acid.

*Mechanism of action of cytochrome c in altering cellular basophilia.* It was noted in Year Books 53 and 54 that treatment of sections of fixed onion-root tips with cyto-

chrome c resulted in a marked decrease in their capacity to stain with basophilic dyes such as pyronin or azure B and an increase in their stainability with acidic dyes such as fast green. Concurrent with the reduction in basophilia was a reduction in absorption of light in the ultraviolet range corresponding to "loss" of purines and pyrimidines (wavelength, ca. 260 m $\mu$ ); there was also an increased absorption at 404 m $\mu$ , showing combination of cytochrome c, or at least its heme component, with the materials of the tissue sections. Similar studies with other proteins (lysozyme, chymotrypsinogen, hemoglobin, egg albumin, and serum albumin) indicated that combination of basic proteins per se with cellular ribonucleic acid was not the primary factor in reducing basophilia; it could account, however, for the increased stainability with acid dyes of tissue sections treated with basic proteins. The results with hemoglobin were essentially similar to those obtained with cytochrome c (see Year Book 54).

Further experiments aimed at determining the mechanisms of these reactions were undertaken this year by McDonald and Sømme. Homogenates of acetic-alcohol-fixed onion-root tips were treated at pH 6 and 37° C with aqueous solutions of ribonuclease, ovalbumin, lysozyme, cytochrome c, hemoglobin, heme, or chymotrypsinogen, or with water, and aliquots were assayed at various time intervals for acid-soluble phosphorus, ribonucleic acid (RNA), deoxyribonucleic acid (DNA), and protein. The results were inconclusive, however, because the high rate of spontaneous autolysis, seen in the controls, obscured whatever augmentation was effected by the added proteins, as is clearly shown in table 5.

*Treatment of growing root tips with ethylenediaminetetraacetic acid.* Treatment of growing onion roots with ribonuclease under certain experimental conditions results not only in the production of mitotic abnormalities (see Year Books 51-53) but

also in the excretion of deoxyribonuclease (Year Book 54). Similar mitotic abnormalities are produced by growing onion roots in solutions of ethylenediaminetetraacetic acid (Year Book 55). In experiments conducted this year by McDonald with this chemical, no release of deoxyribonuclease or of deoxyribonucleic acid was

ases are probably involved. Until they are isolated and characterized, however, their precise role in life processes cannot be definitely established. Attempts were therefore initiated, in 1952, to develop a procedure for the isolation, purification, and crystallization of deoxyribonuclease from calf spleen, a potent source of this group

TABLE 5. Effects of Various Proteins on the Degradation of the Nucleic Acids and Proteins of Homogenates of Fixed Onion-Root Tips \* at 37° C, pH 6  
(Concentration of added protein, 1 mg/ml of homogenate; concentration of homogenate, ca. 100 mg wet tissue/ml)

Treatment	Isoelectric Point of Added Protein	Constituent Measured, per cent of original concentration		
		RNA	DNA	Protein †
H <sub>2</sub> O for 10 hours		85	87	95
H <sub>2</sub> O for 22 hours		65	80	93
Heme for 10 hours		88	87	98
Heme for 22 hours		75	71	97
Ovalbumin for 10 hours	4.7	84	85	99
Ovalbumin for 22 hours	4.7	67	70	98
Hemoglobin for 10 hours	6.8	88	85	99
Hemoglobin for 22 hours	6.8	61	66	98
Chymotrypsinogen for 10 hours	9.5	83	83	97
Chymotrypsinogen for 22 hours	9.5	60	77	99
Cytochrome c for 10 hours	10.7	85	86	96
Cytochrome c for 22 hours	10.7	50	65	94
Lysozyme for 10 hours	10.8	84	88	103
Lysozyme for 22 hours	10.8	41	67	99
Ribonuclease for 10 hours	7.8	9	60 ‡	98
Ribonuclease for 22 hours	7.8	8	28 ‡	97

\* Fixed in acetic alcohol for 24 hours, washed with 70 per cent alcohol, then stored in 70 per cent alcohol for 18 months.  
† Values corrected for added protein.  
‡ This marked decrease in DNA was due to the presence in homogenates of fixed onion roots of a deoxyribonuclease that does not degrade intracellular DNA unless the cells have first been treated with ribonuclease (Year Book 52).

found. There was excreted, however, an unidentified substance, which reacts with Dische's diphenylamine reagent to produce a green compound with an absorption maximum at 650 mμ and a minimum at 550 mμ.  
*Deoxyribonucleases.* Duplication of DNA-containing structures is one of the necessary steps preparatory to cell division. The mechanisms by which the cell synthesizes these macromolecules are not yet known, but intracellular deoxyribonucle-

of enzymes that can be obtained in large amounts at low cost. These endeavors have been continued intermittently since that time. Although a considerable degree of purification of the enzyme has been effected, crystallization has not been achieved (see Year Books 52-54).  
Throughout these experiments we have been hampered by the fact that large quantities of hemoglobin, or its split products, contaminate the enzymatically active fractions and render them gummy and ex-



tremely difficult to manipulate. McDonald has therefore surveyed other tissues in search of a more promising source material. It has been found in salmon testes. Experiments are now being conducted to ascertain the best means of extracting deoxyribonuclease from this tissue and to determine more reliable and reproducible procedures for its assay.

*Separation of tissue cells by crystalline trypsin.* The isolated cells that serve as progenitors for tissue cultures are usually obtained by subjecting compact tissues to the action of the enzyme trypsin. As was demonstrated in our earlier studies, a solution of a salt-free crystalline preparation of trypsin rapidly causes dissociation of the cells of the salivary gland of *Drosophila*. Most of the investigators who have employed this enzyme for the separation of mammalian cells, however, have used crude preparations that were capable of degrading such substances as starch, fats, and nucleic acids as well as proteins. In fact, a survey of the literature reveals that "pancreatin" has been reported more effective than "U.S.P. trypsin," and this in turn more effective than crystalline trypsin. Since at least one of the pancreatic enzymes, ribonuclease, has been proved capable of producing mitotic abnormalities in growing root tips (see Year Books 51-53) and of increasing crossing over in *Drosophila* (Year Book 55), and since one of the main uses for single-cell isolates is the study of spontaneous and induced genetic changes, it seemed advisable to determine whether the active ingredient in crude trypsin preparations is really the enzyme trypsin or some contaminant.

McDonald and Sømme have investigated this problem in co-operation with Dr. Moser. Crystalline trypsin and chymotrypsin, U.S.P. trypsin 1:300, pancreatin ( $3 \times$  U.S.P. potency), and amylopsin ( $2 \times$  U.S.P. pancreatin) were equated on the basis of their ability to degrade denatured hemoglobin. The efficiencies of solutions of

these enzymes having identical proteolytic activity, in obtaining complete cell separation without appreciable loss of viability, were then compared. All the preparations tested, with the exception of chymotrypsin, were found to be powerful agents for the dissociation of mammalian cells. Very efficient separation, with a minimum of toxicity, was obtained with either 0.005 per cent crystalline trypsin, 0.05 per cent U.S.P. trypsin, or 0.25 per cent pancreatin ( $3 \times$  U.S.P.). The toxic effects previously found with crystalline trypsin were evidently due to the use of too concentrated solutions.

*Effect of gibberellic acid on root-tip cells.* Gibberellic acid and its salts (gibberellins) act as growth-promoting substances when applied to plants. The customary method of treatment is to spray a solution of the chemical on the foliage. Some workers have claimed that when plants are immersed in a solution of gibberellin the roots suffer considerable damage. In an effort to determine the action of this chemical agent on root-tip cells, roots of onion bulbs that had been sprouted in sand were immersed in solutions of the potassium salt of gibberellic acid, and the effects were studied cytologically by De and Kaufmann.

In low concentrations, ranging from 1 to 100  $\mu\text{g}/\text{ml}$  (ppm), this chemical had no pronounced effect on the growth of the root or on the mitotic processes in the meristematic region. A concentration of 1000 ppm, on the other hand, markedly altered the appearance of the chromosomes in some of the meristematic cells. In late prophase or premetaphase cells, for example, the chromosomes were greatly elongated, as if partially despiralized, but had enough tight kinks or coils to resemble, at least superficially, the salivary-gland chromosomes of the Diptera (pl. 4, M-O). This type of effect has been described by some cytologists as "erosion," and attributed to the removal of "matrix" material. Cytochemical studies to determine the

exact nature of the changes involved are now in progress. It should be noted that even this highest concentration of gibberel-

lin did not inhibit growth of the roots, which seemed as vigorous as the control roots that had been immersed in water.

## GENETIC AND CYTOLOGICAL STUDIES OF MAIZE

*Barbara McClintock*

The mode of action of controlling elements in maize, a main topic of recent reports, has continued to be studied during the past year. Attention has been directed particularly to the system of elements of which *Spm* (Suppressor-mutator) is a component. This system, which affects the action of genic substances at the  $A_1$  locus in chromosome 3, has been described in previous reports and is referred to as the  $a_1^{m-1}$ -*Spm* system. It has been concluded that  $a_1^{m-1}$  arose by insertion of an element, belonging to the system of which *Spm* is a component, at the standard  $A_1$  locus. This element directly affects the action of the gene substance at  $A_1$ , altering it in a recognizable way, and the modified  $A_1$  locus has been designated  $a_1^{m-1}$ . It responds in readily detectable ways to the presence of the independently located element *Spm*, and one of the responses effects changes in gene action in both somatic and germinal cells. The *Spm* element may undergo change in location within the chromosome complement by a process termed transposition. Methods of detecting transpositions of controlling elements have been discussed in recent publications. One method takes advantage of the linkage relation between the element and a given genetic marker; transposition of the element to a new location alters this relation, and such alterations are easily detected. Two tests utilizing this method were conducted with *Spm* during the year, to reveal more about the degree of stability of its location. They will be summarized below.

The tests determined the numbers of *Spm* elements in different parts of individual plants, and their locations in the chromosome complement. In each test, the

examined individuals represented the progeny of a single plant that carried one *Spm* element at a known location. It was possible to gain some information about the frequency of occurrence of transposition of *Spm* and the period during development when it takes place; but in these respects the results of the two tests were quite different. One gave evidence of frequent transposition of *Spm*, occurring early in development. The other showed infrequent transposition, and thus indicated a considerable degree of stability of location. Although transposition of *Spm* appears to be under genetic control, the factors and conditions associated with it have not yet been recognized.

The first test involved progeny of a plant in which *Spm* was carried in chromosome 9. This plant was  $a_1^{m-1}/a_1$  (chromosome 3), *Wx/wx* (chromosome 9); and it had one *Spm* element in chromosome 9, as the testcross results entered in table 7, A, indicate. Twelve variegated plants, derived from variegated kernels in the *Wx* class of the first ear of this plant (see table 7, A, row 1, column 5), were tested for *Spm* number and location. The silks of all fertile ears produced by each plant received pollen from plants that were homozygous for  $a_1^{m-1}$  and *wx* and had no *Spm*.

Table 6 records the number of ears obtained from each plant and their positions on the plant, the *Spm* constitutions of the cells that produced these ears, and the linkage relations of *Spm* to *Wx*. All together, twenty-six ears were obtained from these 12 plants. In 1 plant, the cells that gave rise to the ear on a tiller (side branch) had no *Spm*, but in the cells that gave rise to the remaining twenty-five ears one or two *Spm* elements were present. In sixteen of



the ears, one *Spm* was present and was linked with *Wx*. In five ears, two *Spm* elements were present and one of them was linked with *Wx*. In four ears, one *Spm* was present, but it was not linked with *Wx*. The kernel types appearing on these three classes of ears are entered in B of table 7. It should be noted that the cells which produced the first and second ears on the main stalk, in the 5 plants from which such ears were obtained, had the

in number of this element in different plants and in different parts of the same plant.

The second test of stability of location of *Spm* was made with the progeny of a plant carrying an *Spm* element located close to *Y* in chromosome 6. This particular location of *Spm* was detected initially only in one plant of a culture. That plant was  $a_1^{m-1}/a_1^{m-1}$ , *Y/y* in constitution; and the silks of one of its ears received pollen from

TABLE 6. *Spm* Constitution and Location in Different Plants of a Culture and in Different Parts of Individual Plants

Plant No. in Culture 7285	No. of Ears Tested per Plant	Position of Ear in Plant	<i>Spm</i> Constitution and Linkage with <i>Wx</i>
A-6, B-1, and B-6.....	1	1st ear, main stalk	1 <i>Spm</i> ; linked with <i>Wx</i>
B-4 .....	1	1st ear, main stalk	2 <i>Spm</i> ; one linked with <i>Wx</i>
A-5 .....	2	1st and 2nd ears, main stalk	2 <i>Spm</i> ; one linked with <i>Wx</i> (both ears)
B-2 and B-5 .....	2	1st ear, main stalk; tiller ear	1 <i>Spm</i> ; linked with <i>Wx</i> (both ears)
A-1 .....	3	1st and 2nd ears, main stalk	1 <i>Spm</i> ; linked with <i>Wx</i>
		Tiller ear	1 <i>Spm</i> ; not linked with <i>Wx</i>
A-3 .....	3	1st and 2nd ears, main stalk	2 <i>Spm</i> ; one linked with <i>Wx</i>
		Tiller ear	1 <i>Spm</i> ; linked with <i>Wx</i>
A-4 .....	3	1st and 2nd ears, main stalk	1 <i>Spm</i> ; not linked with <i>Wx</i>
		Tiller ear	(all three ears)
A-2 .....	3	1st ear, main stalk	1 <i>Spm</i> ; linked with <i>Wx</i>
		Ear on one tiller	1 <i>Spm</i> ; linked with <i>Wx</i>
		Ear on another tiller	No <i>Spm</i>
A-7 .....	4	1st and 2nd ears, main stalk; ear on each of two tillers	1 <i>Spm</i> ; linked with <i>Wx</i> (all four ears)

same *Spm* constitution. In 3 of the 7 plants from which tiller ears were obtained, however, a difference was expressed between the cells of the main stalk and those of a tiller with respect to *Spm* constitution and location.

The results indicate that in the plants of this culture the *Spm* element underwent frequent change of location in the chromosome complement. The time of change was either late in the development of the germinal cells in the parent plant or early in development in the progeny plants. The transposition mechanism will account not only for the changes in location of *Spm* but also for the observed losses or increases

a plant that was homozygous for  $a_1^{m-1}$  and *y* but had no *Spm*. The resulting ear was sectorial, in that a small sector at its base was composed of 47 kernels (21 *Y*:26 *y*) derived from cells in which *Spm* was absent. The cells producing the larger part of the ear carried one *Spm* element. Among the 329 kernels in this part of the ear, 167 had no *Spm*; 10 of them were *Y* and 157 were *y*. The remaining 162 kernels carried *Spm*; 153 were *Y* and 9 were *y*. Close linkage of *Spm* with *Y* was evident, for only 5.6 per cent recombinants appeared among the 329 kernels.

All fertile ears produced by 17 plants derived from the *Y, Spm* class of kernels

TABLE 7. Phenotypes of Kernels on Two Ears of 1 Plant (A), and on Twenty-Five Ears Produced by 12 Plants in Its Progeny (B)

Kernels in A derived from cross of ♀  $a_1^{m-1}/a_1$ ,  $Wx/wx$  × ♂  $a_1^{m-1}/a_1^{m-1}$ ,  $wx/wx$ , no  $Spm$ ; in B, from cross of ♀  $a_1^{m-1}/a_1^{m-1}$  or  $a_1^{m-1}/a_1$ ,  $Wx/wx$  × ♂  $a_1^{m-1}/a_1^{m-1}$ ,  $wx/wx$ , no  $Spm$ .

No. and Location of <i>Spm</i> in ♀ Parent	Phenotype of Kernel						Total No. of Kernels
	Deep Color (germinal mutation)		Pale Color (no <i>Spm</i> )		Colorless with Spots of <i>A</i> <sub>1</sub> ( <i>Spm</i> present)		
	<i>Wx</i>	<i>wx</i>	<i>Wx</i>	<i>wx</i>	<i>Wx</i>	<i>wx</i>	
	<i>Wx</i>	<i>wx</i>	<i>Wx</i>	<i>wx</i>	<i>Wx</i>	<i>wx</i>	
A							
1 <i>Spm</i> ; linked with <i>Wx</i> . . . . .	0	1	26	196	197	18	438
	0	0	38	81	89	29	237
B							
1 <i>Spm</i> ; linked with <i>Wx</i> . . . . .	1	0	418	1539	1512	356	3826 *
2 <i>Spm</i> ; one linked with <i>Wx</i> . . .	0	0	79	267	594	323	1263
1 <i>Spm</i> ; not linked with <i>Wx</i> . . .	0	0	190	168	140	174	672

\* 20.2 per cent are "recombinants."

received pollen from plants that were homozygous for  $a_1^{m-1}$  and  $y$  but had no  $Spm$ . One ear per plant was obtained from 3 plants, two ears per plant were obtained from 4 plants, three ears per plant from 7 plants, and four ears per plant from 3 plants. All together, forty-four ears were produced by these 17 plants, and in all of them the cells that gave rise to the ear carried one  $Spm$ . In forty-three of these

ears, close linkage of  $Spm$  with  $Y$  was expressed; only in the ear produced by a tiller of one plant was there no evidence of linkage of  $Spm$  with  $Y$ . Table 8 records the phenotypes of the kernels that appeared on the forty-four ears.

In contrast to the first-described test, this test revealed a case in which the  $Spm$  element showed a considerable degree of stability of location. As was mentioned

TABLE 8. Phenotypes of Kernels Appearing on Forty-Four Ears Produced by 17 Plants Having the Constitution  $a_1^{m-1}/a_1^{m-1}$ ;  $Wx/wx$  When Pollinized by Plants Homozygous for  $a_1^{m-1}$  and  $wx$  and Having no  $Spm$ 

A. Phenotypes appearing on the forty-one ears produced by 15 of the plants. B. Phenotypes appearing on a partially sterile ear of one plant. Reduction in transmission of the  $Y$ -carrying chromosome is evident. C. Phenotypes appearing on the two ears produced by one plant; (1) main ear, (2) tiller ear.

Ear	Phenotype of Kernel						Total
	$A_1$ (germinal mutation)		Pale Color (no $Spm$ )		Colorless with Spots of $A_1$ ( $Spm$ present)		
	$Y$	$y$	$Y$	$y$	$Y$	$y$	
	$Y$	$y$	$Y$	$y$	$Y$	$y$	
A .....	2	2	247	4708	4551	192	9702 *
B .....	0	0	1	91	20	1	113
C(1) .....	0	0	25	203	171	11	410
C(2) .....	0	0	65	47	48	59	219

\* 4.5 per cent are "recombinants."



earlier, however, nothing is yet known about the genetic or other factors that may control the time during development of a tissue when changes in location of *Spm* will occur, or the frequency of such changes.

### *Types of Spm Elements*

The observed effects of *Spm* control over gene action at  $a_1^{m-1}$  have been remarkably consistent, notwithstanding the various different locations in the chromosome complement the element is known to occupy. Sometimes, however, the action of *Spm* becomes altered, producing modified types of control of  $a_1^{m-1}$  expression. One modification, which arises rather frequently, will be considered here. Occasionally there will appear, on an ear of a plant carrying  $a_1^{m-1}$  and *Spm*, a kernel with an aberrant phenotype. Instead of many deeply pigmented spots in a colorless background, this kernel may have only one or several tiny dots of deep pigmentation in a colorless background. Plants were grown from several kernels of this type, and they and their progeny were examined to determine the nature of the change responsible for the altered phenotype. Tests showed that in such cases an *Spm* element is present, but its capacity to suppress gene action at  $a_1^{m-1}$  and to induce mutation at that locus is much weakened. It has therefore been given the symbol *Spm-w*. In this part of the discussion, the standard *Spm* element will be designated *Spm-s* to distinguish it from the *Spm-w* element.

*Spm-w* elements have been found in several different chromosomes of the complement. The one to be considered here first appeared in a single kernel on an ear produced by a plant carrying *Spm-s* in chromosome 5. This kernel showed only a few dots of deep pigment in a colorless background. The plant that developed from it was pigmented throughout, in contrast to plants carrying *Spm-s*, which have streaks of deep pigmentation in a nonpigmented background. Tests revealed the presence in this plant of an *Spm-w* element, located

in chromosome 5, which showed the same value of recombination with *Pr* as the *Spm-s* element in the parent plant.

Part of the progeny of the plant was examined, in turn, and tests of the different types of individuals it contained made it possible to define the weakened action of the *Spm-w* element. The reduced capacity of *Spm-w* to suppress gene action at  $a_1^{m-1}$  is shown by the appearance of anthocyanin pigment throughout plants that carry it. The development of this pigment, however, is very much retarded in comparison with that in plants that have no *Spm* element. Plants with *Spm-w* become fully colored only at late maturity, whereas plants with no *Spm* develop pigment at early stages. The weakened capacity of the *Spm-w* element to induce mutation at  $a_1^{m-1}$  is also shown by the phenotypes of kernels that carry it. Either mutant spots are totally absent, or just one or a few spots appear.

Table 9 has been constructed to show the linkage of this *Spm-w* element with *Pr*. When both *Spm-s* and *Spm-w* are present in the same plant, *Spm-s* is epistatic to *Spm-w*. Both elements segregate normally at meiosis, however, as illustrated by the kernel types on the ears of the testcross recorded in table 10. Each of the 5 plants used in this testcross carried *Spm-s* in chromosome 6, closely linked with *y*, and *Spm-w* in chromosome 5, linked with *Pr*.

Another type of *Spm* element has received preliminary examination, but description will be postponed until more is known about its action. *Spm* elements with altered expressions may represent changes in state of the standard *Spm-s* element. If so, their origins should be comparable to the changes in state of *Ac*.

### *A Modifier Element within the Spm System*

An element which greatly increases the rate of mutation at  $a_1^{m-1}$  in the presence of *Spm* first appeared in a kernel on one ear of a plant that was  $a_1^{m-1} Sh_2/a_1 sh_2$

TABLE 9. Phenotypes of Kernels Appearing on Ears Produced by 20 Plants from the Cross  
♀  $a_1^{m-1}/a_1^{m-1}$ ;  $Pr\ Spm-w/pr \times \sigma a_1^{m-1}/a_1^{m-1}$ ;  $pr/pr$ ; no  $Spm-w$ 

Phenotype of Kernel with Regard to $a_1^{m-1}$ Action	$Pr$	$pr$	Total	Per Cent Recombinants
Pale color (no $Spm-w$ ).....	1016	2943	3959	25.6
Colorless with one or several $A_1$ dots or specks ( $Spm-w$ present) .....	1780	569	2349	24.2
Colorless; no $A_1$ specks ( $Spm-w$ present).....	.....	.....	1462	....
Total number of kernels.....			7770	
Total with $Spm-w$ .....			3811	

in constitution and carried one  $Spm$  element. The silks of two ears of this plant received pollen from a plant that was homozygous for  $a_1$  and  $sh_2$  and had no  $Spm$ . These two ears produced a total of 738 kernels; 354 had  $a_1^{m-1}$  and  $Sh_2$ , and 384 were homozygous for  $a_1$  and  $sh_2$ . Of the kernels in the  $a_1^{m-1} Sh_2$  class, 167 were uniformly pale colored (no  $Spm$ ) and 187 had spots of deep color in a colorless background ( $Spm$  present). All but one of the variegated kernels exhibited the number of mutant spots that is characteristically produced by the state of  $a_1^{m-1}$  known to be present in the pistillate parent. One kernel, however, had a much larger number of mutant spots; and the plant grown from it showed a marked increase in number of streaks of deep pigmentation in a non-

pigmented background. To investigate the reason for this altered expression of  $a_1^{m-1}$ , the plant was self-pollinated, was crossed reciprocally with a plant that had another state of  $a_1^{m-1}$  but no  $Spm$ , and was used as a pollen parent in crosses with plants that were homozygous for  $a_1$  and  $sh_2$  and had no  $Spm$ . The tests revealed the presence in this plant of an unmodified  $Spm$  element, and also an unmodified  $a_1^{m-1}$  locus. It had an independently located element, however, which was capable of markedly increasing the frequency of occurrence of mutation at  $a_1^{m-1}$  when  $Spm$  was also present; and both states of  $a_1^{m-1}$  responded to it in this manner. Plants were grown from the various classes of kernels appearing on each of the ears produced by the above-indicated crosses; and they, in

TABLE 10. Phenotypes of Kernels Appearing on Ears of 5 Plants Produced by Cross of  
♀  $a_1^{m-1}/a_1^{m-1}$ ;  $Y/y\ Spm-s$  (standard  $Spm$ );  $Pr\ Spm-w/pr \times \sigma a_1^{m-1}/a_1^{m-1}$ ;  
 $y/y$ ;  $pr/pr$ ; no  $Spm-s$ ; no  $Spm-w$ 

Phenotype of Kernel with Regard to $a_1^{m-1}$ Action	$Y\ Pr$	$Y\ pr$	$y\ Pr$	$y\ pr$	Totals
Pale color (no $Spm-s$ , no $Spm-w$ ).....	98	200	9	12	319
Many $A_1$ spots in colorless background ( $Spm-s$ present) .....	16	19	360	342	737
One or several $A_1$ dots or specks in colorless background ( $Spm-w$ , no $Spm-s$ ).....	212	69	11	9	301
Colorless; no $A_1$ dots or specks ( $Spm-w$ , no $Spm-s$ ).....	56 Y		5 y		61
Total kernels .....					1418

Summaries: 737  $Spm-s$  (376  $Pr$  : 361  $pr$ ; 35  $Y$  : 702  $y$ )  
 362  $Spm-w$ ; no  $Spm-s$  (223  $Pr$  : 78  $pr$ ; 337  $Y$  : 25  $y$ )  
 319 no  $Spm-s$ , no  $Spm-w$  (107  $Pr$  : 212  $pr$ ; 298  $Y$  : 21  $y$ )  
 29.8% recombination between  $Pr$  and  $Spm-w$   
 5.7% recombination between  $y$  and  $Spm-s$



turn, were tested for presence and absence of the modifier element, and for its inheritance behavior when present. These tests not only confirmed the conclusions drawn from tests of the parent regarding the presence and mode of action of the modifier element; they also showed that the element could undergo change of location in the chromosome complement in somatic cells. In this respect, therefore, its behavior is much like that of some of the other controlling elements.

The origin of this new controlling element, in the system of which the *Spm* element and the element at  $a_1^{m-1}$  are components, is not understood. It is known, however, that without the new element the types of mutation, their time of occurrence during development of a tissue, and their frequency of occurrence are expressions of the state of  $a_1^{m-1}$  itself. The modifier element affects the expression of only one of these three aspects of state, namely, the frequency of occurrence of mutation. When it is present in conjunction with one of the states, the increase in mutation frequency is estimated to be about threefold.

#### *The Relation between $a_1^{m-1}$ and $a_2^{m-1}$*

It has recently been determined that the system of elements responsible for control of gene action at  $a_1^{m-1}$  also operates to control gene action at  $a_2^{m-1}$ . In this respect, the history of origin of both  $a_1^{m-1}$  and  $a_2^{m-1}$  is of considerable significance, and so will be outlined briefly.

Some years ago, in the progeny derived from self-pollination of a plant, a number of individuals exhibited variegation in leaf and sheath with regard to intensity of chlorophyll pigmentation. A study was commenced to examine the expression of this variegation and also its inheritance pattern. In the course of study, many plants in one culture that carried the control system regulating chlorophyll expression were self-pollinated. On the ear produced by one of these plants, some kernels exhibited variegation for anthocyanin pigmentation.

Plants derived from the variegated kernels also showed variegation for anthocyanin pigmentation, and tests conducted with them made it possible to associate this phenotypic expression with an alteration that had occurred at the standard  $A_2$  locus in one chromosome 5 of the parent. The modified locus was designated  $a_2^{m-1}$ , for in kernels carrying it spots of anthocyanin appeared in a colorless background, as if the change in gene expression was from recessive to higher alleles of  $A_2$ .

Study was continued, and in its course the silks of an ear of a plant carrying the system responsible for control of mutations at  $a_2^{m-1}$  received pollen from a plant that was homozygous for  $a_1$ , carried in chromosome 3, and for the standard  $A_2$  locus, carried in chromosome 5. On the resulting ear, one exceptional kernel, instead of being totally pigmented, had spots of deep pigmentation in a colorless background. The plant derived from this kernel also exhibited variegation for anthocyanin pigmentation. Tests of the plant indicated the presence of the  $a_1$  locus derived from the male parent and of an altered  $A_1$  locus derived from the female parent. Alteration of the  $A_1$  locus must have occurred late in development of the ear of the pistillate parent plant, for only this one kernel exhibited modified  $A_1$  action. The modified locus was designated  $a_1^{m-1}$ . All studies of  $a_1^{m-1}$  have been made with progeny of this single plant.

Although similarities in expression were noted between the chlorophyll variegation originally studied and the variegations associated with the modified  $A_2$  and  $A_1$  loci ( $a_2^{m-1}$  and  $a_1^{m-1}$ ), investigation of the operation of the systems responsible for control of gene action in the first two cases was suspended. Attention was concentrated instead on examination of the system responsible for control of gene action at  $a_1^{m-1}$ . When the mode of operation of that system, the *Spm*- $a_1^{m-1}$  system, was appreciated, it became clear that further consideration should be given to  $a_2^{m-1}$ , to

determine whether or not changes in expression of genic materials at this locus are under the control of elements belonging to the same system. Pedigree relationships as well as kinds of behavior suggested such a possibility. Study of the system operating at  $a_2^{m-1}$  was therefore resumed, with this viewpoint in mind.

Two strikingly different states of  $a_2^{m-1}$  were selected for the renewed investigation. Both states respond to an independently located element that exhibits a Suppressor-mutator (*Spm*) type of control of gene action. With one of these states, some gene action occurs at the  $a_2^{m-1}$  locus in the absence of *Spm*, resulting in the appearance of anthocyanin in both kernel and plant, the pigment being uniformly distributed within a tissue. The rate of action appears to be lower than that of the standard  $A_2$  gene, for in both plant and kernel the pigment intensity is low. In the presence of the *Spm* element, however, gene action is suppressed except in some cells where mutations at the  $a_2^{m-1}$  locus, initiated by *Spm*, allow the gene substance to be fully active. These mutations result in stability of expression of the genic materials at the locus in subsequent cell and plant generations. The characteristics of this state of  $a_2^{m-1}$  are essentially similar to those of some states of  $a_1^{m-1}$ .

The expression of the second selected state of  $a_2^{m-1}$  has not yet been satisfactorily analyzed, but it clearly differs from all the many known states of  $a_1^{m-1}$ . In the absence of the *Spm* element, it gives rise to deeply pigmented kernels and plants, although the intensity of color is not so great as that produced by the standard  $A_2$  locus. When the *Spm* element is present, both kernel and plant are variegated. Pigmented areas in the kernel have about the same intensity as that produced in the absence of *Spm*; but colorless areas may be present within the pigmented areas. The pattern of variegation in any one kernel depends upon the number of *Spm* elements present. When only one *Spm* element is present,

the kernels have many large pigmented areas as well as some small pigmented spots. When two *Spm* elements are present, there are few if any large pigmented areas, and most of the kernels have only small pigmented spots. If three or more *Spm* elements are present, the kernels either are totally colorless or have small pigmented spots in a restricted region of the aleurone layer. The type of pigment in the colored areas, and the observed effects of dose of *Spm* on the pattern of their appearance, suggest that the variegation in this case is not related to mutation at the  $a_2^{m-1}$  locus, as it is with the previously described state of the locus. Rather, the pigmented areas seem to reflect changes affecting the *Spm* element, which either result in its removal or alter its capacity to suppress gene action with this state of  $a_2^{m-1}$ .

The *Spm* element that was present in the cultures having the two states of  $a_2^{m-1}$  is not the same as the *Spm-s* element carried in the  $a_1^{m-1}$  cultures. Changes in its mode of control of gene action at  $a_2^{m-1}$  sometimes occur in somatic cells, and these are often expressed in different sectors of the same plant. They affect both the suppressor and the mutator action of the *Spm* element. The nature of these changes is not yet understood; but the marked difference in stability of action between this *Spm* element and the *Spm-s* element was strikingly revealed when the latter was introduced into an  $a_2^{m-1}$  culture. The *Spm-s* element exerts a consistent and stable control of gene action at  $a_2^{m-1}$ , of a type similar to that which it exerts at  $a_1^{m-1}$ .

On the other hand, the mode of inheritance of the *Spm* element in the  $a_2^{m-1}$  cultures is similar to that in the  $a_1^{m-1}$  cultures. Somatic occurring transpositions bring about loss of *Spm* from some cells, change of its location in others, or changes in both number and location. The several methods adopted to detect such transpositions in the  $a_1^{m-1}$  cultures have also been applied here. In addition, use of the state of  $a_2^{m-1}$



that reflects doses of *Spm* has made it possible to select among the kernels on an ear those that have different numbers of *Spm* elements. The effectiveness of this selective method was shown by tests of 12 plants (group A) derived from kernels whose variegation patterns indicated the presence of more than one *Spm* element, and 10 plants (group B) derived from kernels whose variegation patterns indicated the presence of only one *Spm* element. One of the plants in group A had no *Spm*, which suggested that transposition, occurring in the gametophyte, had resulted in increase in number of the *Spm* element in the endosperm nucleus and its absence in the zygote nucleus. In tests of the remaining 11 plants, one testcross ear per plant was obtained from 5 plants, two testcross ears per plant from 5 others, and three testcross ears from the eleventh. In 1 plant, three or four *Spm* elements were present in the cells that gave rise to an ear on the main stalk and one on a tiller. Seven plants had two *Spm* elements in the cells that produced the main and the tiller ears. In the remaining 3 plants, two *Spm* elements were present in the cells that gave rise to the main ear, but only one *Spm* element in the cells that gave rise to a tiller ear.

Among the 10 plants in group B, one testcross ear per plant was obtained from 2 plants, two testcross ears per plant from 5 plants, three testcross ears from 1 plant, and four testcross ears per plant from the remaining 2 plants. All the ears, except two tiller ears of 2 plants, were produced from cells having one *Spm*. The cells that produced each of these tiller ears had two independently located *Spm* elements.

The finding that the same system of elements controls gene action at  $a_1^{m-1}$  and at  $a_2^{m-1}$  is not unexpected. Similar relationships with respect to control of gene action have been observed at a number of different loci in cultures carrying the *Ds-Ac* system. Insertions of the *Ds* element at different gene loci have initiated control

of action of the genic substance at each locus by this system of elements. The origin of  $a_1^{m-1}$  by modification of a standard  $A_1$  locus in a culture carrying the elements responsible for control of gene action at  $a_2^{m-1}$  suggests that at both loci an element of common ancestry is present. That element may also have been present at the locus of the gene responsible for chlorophyll variegation, for the chlorophyll variegate was the first-recognized member of this sequence of gene change. The possibility cannot be tested, however, since the cultures carrying it were discarded some years ago.

It is also not unexpected to find differences in expression of the *Spm* element in the  $a_1^{m-1}$  and  $a_2^{m-1}$  cultures, for a series of selections was made among the  $a_2^{m-1}$  cultures before the system responsible for control of  $a_2^{m-1}$  action was recognized. We know, too, that changes may occur in the action of the *Spm* element in  $a_1^{m-1}$  cultures, as described previously. Moreover, other elements may appear, such as the modifier of rate of mutation at  $a_1^{m-1}$  discussed above; and these, if their presence is not recognized initially, may be responsible for unwitting bias in the selection of kernels and plants for subsequent study. In this connection, it is suspected, although not yet certain, that an inhibitor of *Spm* may be present in some of the  $a_2^{m-1}$  cultures.

Obviously, since the number of variables may increase during the course of a study, analyses of systems of controlling elements can sometimes be complicated and time consuming. Recognition of the different elements belonging to a control system, and of the changes that may occur in them as regards both type of action and location in the chromosome complement, requires many types of test. In order to study any one element of a system, each of the other variables, as it is recognized, must be removed by crossing and selection, so as to work with the smallest possible number of associated and interacting elements.

### *Aberrant Behavior of a Fragment Chromosome*

Much effort has been expended during the past year in analysis of a structurally modified chromosome 9, of which preliminary investigations were reported in Year Book 55. In this modification the substance of chromosome 9 is distributed between two components. The distal third of the short arm comprises one member, referred to as the fragment chromosome. At the proximal end of this segment is a centromere, from which may extend a small, deeply staining piece of chromatin. The extension is often lost, however, leaving the fragment with a terminal centromere. The other component carries the proximal two-thirds of the short arm and all of its long arm, and is referred to as the deficient chromosome. The fragment chromosome shows aberrant behavior in somatic cells. It may be lost to some cells, and undergo changes in structural organization in others. It may also become attached to ends or centromeres of other chromosomes, or be incorporated into another chromosome. Both the frequency of occurrence of events leading to such consequences and the time of their occurrence during the development of a tissue are now known to be under genetic control.

Interest in this structurally modified chromosome was aroused initially by the aberrant behavior of the fragment chromosome in somatic cells. Later it was discovered that this fragment could behave unexpectedly in some of the meiotic cells also, and in plants either heterozygous or homozygous for the structural modification. Although there is no conspicuous cytological evidence of the fact, it has been shown genetically that a segment of chromatin, adjacent to the centromere in the

fragment chromosome, duplicates a segment at the end of the deficient chromosome. Products that could be assumed to arise from crossing over between this segment in the fragment and the homologous segment in the normal or in the deficient chromosome have appeared in the progeny of both heterozygote and homozygote. It is certain, however, that not all of them result from the ordered process of meiotic events that normally leads to crossing over; and this is made particularly evident in the apparent crossover products formed at meiosis in the homozygote. Often these are structurally normal chromosomes 9, but sometimes they are defective. The frequency of appearance of crossover products in the gametes of the homozygote varies widely among the different homozygotes, but is constant for any one of them. The present evidence, though limited, does suggest that the variation may be an expression of the genetic system that controls the time of occurrence of aberrant events altering the organization of the fragment chromosome. If so, then when this system operates in a meiotic cell the crossover mechanism may be utilized but the fragment chromosome itself may not be required to undergo the usual preliminaries that normally control the position of crossing over and its frequency of occurrence at any one position. At any rate, it is certain that the rules assumed to apply to crossing over in maize are not always followed by the fragment chromosome when it participates in a crossover type of event.

Until more definite conclusions can be drawn regarding the mechanism responsible for the complicated behavior of the fragment chromosome, a review of the evidence obtained from the many tests conducted with it will be postponed.



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## PERSONNEL

Year Ending September 30, 1957

- |   |   |
|---|---|
| Buchanan, Jennie S. (Mrs.), Research Assistant                | Demerec, M., Director                       |
| *Burgi, Elizabeth, Associate in Research                      | *Djordjević, Božidar, Research Assistant    |
| Carley, Catherine, Switchboard Operator and Computer          | *Fillekes, John R., Maintenance Man         |
| *Clowes, Royston C., Fellow of the Damon Runyon Memorial Fund | Fisher, Agnes C., Secretary to the Director |
| *De, Deepesh Narayan, Research Assistant                      | Gay, Helen, Associate in Research           |
|   | Goldman, Irving, Research Assistant         |
|   | Gross, Julian D., Research Assistant        |
|   | Haggerty, Michael J., Maintenance Man       |

Hashimoto, Kazuo, Carnegie Institution Fellow

Hershey, Alfred D., Microbiologist

\*Howarth, Sheila, Associate in Research

Jones, Henry H., Photographer

\*Käfer, Etta, Carnegie Institution Fellow

Karossa, Judith, Research Assistant

Kaufmann, Berwind P., Cytogeneticist

\*Kelly, Kathleen R., Stenographer

Kozinski, Andrej W., Fellow of the Polish Academy of Sciences

Lahr, Ernest L., Associate in Research

McClintock, Barbara, Cytogeneticist

McDonald, Joseph L., Janitor

McDonald, Margaret R., Chemist

McDonald, William T., Janitor

McIntyre, Jean W. (Mrs.), Technical Assistant

Mandell, Joseph D., Carnegie Institution Fellow

\*Martinello, Marian L., Research Assistant

Meissner, Richard C., Superintendent of Buildings and Grounds

Miyake, Tadashi, Research Assistant

\*Ozeki, Haruo, Research Assistant

Peckham, Leslie E., Senior Clerk

Rogers, Claude F., Chief Clerk

\*Sengün, Atif, Carnegie Institution Fellow

Sepe, Domenico, Greenhouse Man

Smith, Guinevere C. (Mrs.), Librarian, Curator of *Drosophila* Stocks

Snyder, Emmy M. (Mrs.), Technical Assistant

\*Sømme, Randi (Mrs.), Research Assistant

Streisinger, George, Associate Geneticist

Thomas, René Paul-Émile, Rockefeller Foundation Fellow

Tomizawa, Jun-ichi, Associate in Research

Van Houten, William B., Engineer

Wassermann, Felix, Guest Investigator

White, Harry, Chief Mechanic

Wilson, Carole E., Technical Assistant

Yoshida, Yoko, Research Assistant

### *Summer 1957 and Temporary*

Anderson, Richard P., Maintenance Man

Baer, Harold, Guest Investigator

Beckwith, Barbara, Assistant

Bert, Grace R., Assistant

Burtch, Ethel P. (Mrs.), Typist

Cannon, W. Dilworth, Jr., Assistant

Fochtman, Grace M., Assistant

Gots, Joseph S., Guest Investigator

Gregory, Jack, Assistant

Holden, Floyd, Maintenance Man

Nevole, Nancy A., Assistant

Page, Gilbert, Maintenance Man

Powell, Florence (Mrs.), Assistant

Simrell, Elizabeth Jane, Assistant to Librarian

Starfield, Phoebe D., Assistant

Streisinger, Lotte, Assistant

Victoria, William, Maintenance Man

### *Collaborators at Biological Laboratory*

Barratt, R. W., Summer Investigator

Bernheimer, Alan W., Summer Investigator

Calef, E., Summer Investigator

Caspari, E. W., Summer Investigator

Englesberg, Ellis, Bacteriologist

Errera, M., Summer Investigator

Franzese, Eleanor, Business Manager

Granick, S., Summer Investigator

Hotchkiss, Rollin D., Summer Investigator

Hyde, Olive, Administrative Assistant

King, James C., Geneticist

Luria, S. E., Summer Investigator

Maramorosch, Karl, Summer Investigator

Novick, A., Summer Investigator

Skaar, Palmer D., Bacterial Geneticist

Wallace, Bruce, Assistant Director, Geneticist

Watanabe, T., Summer Investigator

Witkin, Evelyn M., Summer Investigator, Instructor

\* Resigned during the year.





# DEPARTMENT OF ARCHAEOLOGY

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*Cambridge, Massachusetts*

H. E. D. POLLOCK, *Director*



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## GENERAL

The work of the year under review has followed the pattern indicated in our previous annual report (Year Book 55). With the completion of field activities, the efforts of the staff have been directed to the production of preliminary reports on the field work and to the broader studies that will lead to definitive statements covering the results of our recent program of researches in Yucatan.

Our progress in the first area is recorded here in a subsequent section dealing with publications. This phase of the work is now virtually completed, only two or three such reports remaining to be published.

In connection with the larger studies R. E. Smith worked in Yucatan, from the beginning of December 1956 until the end of May, with the large collections of pottery stored in our Merida headquarters. Because of the vast amount of material of this nature that was produced in our five seasons of excavations at Mayapan and elsewhere in the Yucatan Peninsula, this study is the most time-consuming of the several major divisions of our researches. A statement of Smith's progress is contained in the following section of this report.

Thompson made a trip to Yucatan in March 1957 for study of the effigy incense burners recovered from Mayapan, to determine what light they would throw on the religion of Mayapan and of the late preconquest Yucatecan Maya in general. The results of this work were highly rewarding, in that he was able not only to identify a number of the gods or personages represented on the incense burners and to make tentative identification of others, but also to show how considerably the religion of Mayapan was influenced by foreign ideologies. A more detailed report on this work will be found below.

A. L. Smith has continued his research dealing with the domestic architecture and living patterns of the people of Mayapan.

The raw data for this study are extensive, and their ordering and analysis are of necessity a slow, painstaking process. The work has progressed from the study of types of individual structures to the examination of groups of buildings that presumably housed family units. It is too early as yet to anticipate the results of these studies.

Proskouriakoff has been engaged in preparing reports on the formal architecture of Mayapan and the artifact remains, other than pottery, from that site. In regard to architecture, her careful work has developed some interesting information concerning assemblages of specific types of structures that may have functioned as ceremonial units. It has also brought out the necessity for changing our ideas in regard to the type, and presumably the function, of certain buildings. A highlight of the study of artifacts is the suggestion that the date of the introduction of metal into Yucatan may have been later than it has commonly been thought to be.

During the past year Shook has been on leave of absence from the Institution. As was stated in our last report, he has been in charge of the work of the University Museum, University of Pennsylvania, at the ruins of Tikal in Guatemala.

Shepard has continued her academic work in geology in anticipation of making that her principal field. Certain of her studies, especially theoretical igneous petrology, metamorphic petrology, ore genesis, paragenesis, and clay mineralogy, have been of direct benefit in her research in ceramic technology.

In addition to her class work and a review of the literature of clay mineralogy, Shepard has investigated Maya pigments, particularly *Maya blue*—an exceptional pigment that has so far defied identification. In collaboration with Dr. R. J. Gettens, of the Freer Museum, Smithsonian Institution, Shepard has examined petro-



graphically a number of blue minerals obtained from the U. S. National Museum through Dr. Gettens. She has also run thermal tests and made some experiments in synthesis. She has now arranged for a microorganic analysis, the results of which, together with results of a semiquantitative spectrographic analysis being supplied Dr. Gettens by the U. S. Geological Survey, should define the composition of the pigment.

During February and March 1957, the Director had the opportunity to accompany Dr. Gordon R. Willey and Dr. Philip Phillips, of the Peabody Museum, Harvard University, on a visit to certain ruins, museums, and private collections in Mexico. The journey extended from the northern border of the country to Yucatan, most of the distance being traversed by automobile. Quite aside from the archaeological remains, a large part of which were seen for the first time by the Director, he was reminded of the value of first-hand knowledge of the environment in which the various aboriginal cultures existed. The trip was a most stimulating experience.

As has been mentioned in earlier reports, the Department has frequently relied upon the aid of scholars in fields other than archaeology. Shepard's collaboration with Dr. R. J. Gettens on Maya pigments has been referred to above. The work of Dr. W. C. Root on the analyses of metals recovered from Mayapan, noted in last year's annual report, has continued. We are indebted to Professor Hessel de Vries, of the Natuurkundig Laboratorium der Rijks-Universiteit te Groningen, Holland, for another carbon-14 age determination of charcoal from Mayapan. The specimen was selected because of its important archaeological associations. It marks a late phase in the history of Mayapan. The sample, GRO 1166, gave a reading of 400 (A.D. 1557)  $\pm$  55 years. Allowing a value of 100 to 200 years for "Suess effect" brings the date closely in line with historical evidence that Mayapan was abandoned about A.D. 1450. (A similar correction should be

applied to the dates published in Year Book 55, p. 336.) Lastly, the Department has been instrumental in obtaining for Professor W. Sandermann, of the Institut für Holz- und Zellstoffchemie, Reinbek bei Hamburg, Germany, samples of contemporary tropical woods to use in connection with his identifications of ancient woods from Maya ruins.

In anticipation of the closing of this Department a year hence, we have continued to dispose of certain records and equipment no longer needed. Gifts of this sort have been made to the Museo Nacional de Arqueología y Etnología of Guatemala, the Instituto Nacional de Antropología e Historia of Mexico, the University Museum of the University of Pennsylvania, the University of Arizona, the University of Colorado, and the Haddon Library of Cambridge University, England.

At the Twenty-Second Annual Meeting of the Society for American Archaeology, held in May 1957 at Madison, Wisconsin, the Department was represented by Thompson, who presented the paper, "Divinatory Almanacs and Hieroglyphs for Diseases in the Maya Codices: A New Discovery." As is indicated by the title, the findings set forth in this paper represent a real advance in the reading of previously undecipherable hieroglyphs.

In October 1956 the Director attended an international Conference on Radiocarbon Dating at Andover, Massachusetts, sponsored by the R. S. Peabody Foundation and the National Science Foundation. During the same month the Director participated in the first meeting of the Committee for Latin American Anthropology of the National Research Council, held at the Wenner-Gren Foundation, New York. The purpose of the meeting was to discuss plans for the preparation and publication of a handbook of Middle American Indians. In February 1957, Dr. Gordon R. Willey, Chairman of the above committee, and Pollock met with a number of Mexican colleagues at the Museo Nacional de

Antropología in Mexico City for further discussion of the same subject.

Karl Ruppert, the senior member of the staff in length of service, retired on October 1, 1956. His retirement terminated an association of almost thirty-two years. Coming to the Institution early in 1925, Ruppert did his first work under the late E. H. Morris in his excavation and restoration of the Temple of the Warriors at Chichen Itza. Ruppert's talent for the complicated procedures involved in creating authentic restoration of the intricate stone architecture of the Maya soon became apparent, and most of his time for the next dozen years was devoted to work of this sort. At Chichen Itza the Temple of the Wall Panels, the Caracol, the Mercado, and the Sweat House stand as monuments to his talent.

Although mainly occupied in these early years with architectural restoration, Ruppert had already embarked upon some of the explorations that were to characterize the next phase of his career in Middle American archaeology. By 1936, when the rebuilding of the Sweat House at Chichen Itza was completed, he had traveled overland to the east coast of the Yucatan Peninsula, to the ruins of Yaxchilan on the Usumacintla River, and had made three trips into the virtually unexplored regions of southeastern Campeche and southwestern Quintana Roo. In the next six years his travels took him to southern Veracruz, where he worked in co-operation with archaeologists of the Mexican government, to southern Chiapas, again into Campeche, and as far as Nicaragua. After the interruption of the war years, which brought overseas service, Ruppert was soon again embarked upon difficult explorations, now to eastern Chiapas and once more to Campeche.

With the beginning of the Department's recent operations at Mayapan, Ruppert and A. L. Smith joined in the tremendous task of recording the several thousand dwelling-type structures at that site and in the immediately surrounding area, extending

their survey to include comparative material from other areas in Yucatan. It is safe to say that the records accumulated by these men comprise the largest body of information on the domestic architecture of the Maya now in existence. It was the analysis of this material, data that will be used by Smith in a forthcoming monograph, that engaged Ruppert up to the time of his retirement. His wide experience and friendly help will be missed.

At the close of the period under review, Gustav Strömsvik, second only to Ruppert in length of service, retired. His association with the Institution, beginning early in 1926, covered a span of more than thirty-one years. Strömsvik's abilities proved to be so varied that he was called upon to perform many tasks not strictly archaeological in nature. In his early years at Chichen Itza he assisted in building the then growing camp, and for a number of years was responsible for the operation of all mechanical equipment there, a task requiring no small ingenuity because of limited supplies and facilities. He worked with Morris on the difficult engineering problem of supporting an overlying temple while one beneath it was being excavated. In 1934, first working under Morris but later in charge, Strömsvik re-erected, repaired, and stabilized a number of the huge monolithic monuments at the ruins of Quirigua in Guatemala, an achievement that quickly led to his being called upon to do similar work at Copan, Honduras, and to carry out the even greater task of deflecting the Copan River from eroding away the magnificent acropolis at that site. Between archaeological field seasons he built and installed a small museum in the village of Copan, and supervised the construction of an aqueduct to serve the village, thus providing a public facility beyond the reach of the community without his assistance.

Strömsvik's archaeological activities have covered a wide range. In his early years in Yucatan, he participated in expeditions to Quintana Roo and southern Campeche.



During the summer and fall of these years, when excavation was at an end in the tropics, he worked with Morris in the Southwest of the United States. In 1933 he carried out the work of consolidating a part of the building known as the Temple of the Phalli at Chichen Itza. His work at Quirigua, already mentioned, included excavation and the making of plaster casts of some newly discovered and unusually fine monuments there. At the magnificent ruins of Copan, where from 1935 to 1942 he was in charge of the co-operative undertaking by the Institution and the Government of Honduras, the entire valley was explored and mapped, large-scale plans and sections were made of the ruins, and Temples 11 and 22, the Ball Court, and the Hieroglyphic Stairway were partly restored. The result of this work was the declaring of the ruins a national park by the Government of Honduras.

Strömsvik served with the Norwegian Navy during World War II. Upon his return from that duty he soon was occupied with a variety of archaeological assignments. In 1946 he assisted the United Fruit Company operations at Zaculeu in Guatemala; in 1947 and 1948 he participated in the United Fruit Company fi-

nanced Carnegie Institution-Mexican Government expeditions to Bonampak, Chiapas, being in charge the latter year; in 1949 he carried out an archaeological reconnaissance at the ruins of Asuncion Mita in Guatemala. With the beginning of our recent operations in Yucatan, Strömsvik saw to the purchase and supply of the new equipment needed and supervised the construction of our field headquarters. During five seasons of operations there he was responsible for the proper functioning of all equipment and at the same time had charge of our program of stabilization of a limited number of structures in the ruins of Mayapan. He also participated in expeditions to southern Yucatan and into Quintana Roo, and carried out exploratory work in the Cave of Dzab-Na, Tecoh, Yucatan. Lastly, it fell to his lot to dismantle our field camp at the close of our operations in Yucatan.

Few careers have been more varied. No task has been too large to undertake, no task too small to deserve his attention. In his native Norway, where Strömsvik has retired, he plans to occupy himself with archaeological problems of that land. His colleagues and his host of friends on this side of the Atlantic wish him well.

## CERAMIC STUDIES IN YUCATAN

*R. E. Smith*

During the past winter and spring, work was continued on the extensive collection of pottery from Mayapan. A year ago the study of the surface material was largely completed, including a separation and analysis of the pottery from what we are calling the Late Mayapan Ceramic Phase. Besides the late phase, an early and a middle phase of the Mayapan Ceramic Period (ca. A.D. 1200-1450) appear to exist, and it was in their study that most of the time was spent.

It is interesting to review how the phases were determined in view of the rather inferior stratigraphic conditions at Mayapan: the scarcity of refuse dumps, none of which

represents more than a single phase; the dearth of deep deposits; and the lack of marked architectural change. In order to determine the ceramic changes and therefore the possible ceramic phases, the material was separated into three groups according to level: the uppermost or surface, the middle, and the lowest.

The uppermost level included material found in the humus or surface soil, on floors of rooms, or in fallen debris. Naturally, the pottery in the fallen debris is not necessarily contemporary with that found on the floors of rooms, but since these two groups are often hard to separate, and since no ceramic differences were noted, it was

decided to lump them together as surface lots. Included in the surface lots is found a small percentage of earlier types, some of which came from fill (taken from early refuse deposits) mixed with fallen debris, some being early types still used, although in small quantities.

In separating the pottery of the lowest levels from the rest it was apparent that not all the lowest-level lots were typologically early; some might even belong with latest levels as far as the material was concerned. Therefore, in determining what was a truly early lowest level, we had to review the pottery to make certain that no late ceramic traits were present.

The middle lots include all those not considered surface or lowest and early. In this middle collection of potsherds we find Early as well as Late Mayapan Period traits. The early traits are found in smaller percentages, since they were gradually being discarded to make way for the late traits which were just coming in and which in the surface levels become predominant. The middle phase is essentially transitional.

The Mayapan Ceramic Period, believed to be approximately 250 years long, is represented throughout by two principal wares: Mayapan Red and Mayapan unslipped porous gray or cinnamon. The red has minor shape changes from early to late and a few forms identified specifically with either the early or late phases. There may be typical middle-phase forms so far not identified. The story in the unslipped group is similar to that of the slipped red. Minor wares also play their part. Black-on-cream ware is well represented in the early, diminishes considerably in the middle, and disappears in the late phase. Cream ware follows much the same pattern. Red-on-buff and Red-and-black-on-buff, which may be considered subtypes of Mayapan Red, appear first in the middle but are most prolific in the late phase. There are other minor wares, of which some may be local but most are probably from trade. In

the last category the most important is V Fine Orange, first noted in the middle but most abundant in the late phase.

In all levels, occasionally even in the surface lots, pre-Mayapan Period pottery occurs, including Puuc, Toltec-Chichen, and Peten-like Classic types. The appearance of these early types, mixed with material found in Mayapan Period levels, strongly suggests that they were taken from early deposits and refuse dumps and used for fill.

Another task completed this past season was the recording and analysis of the pottery from 9 of the 19 cuts dug at Chichen Itza in 1954 by E. M. Shook and R. E. Smith. The three objectives advanced in the report (Year Book 53, pp. 286-289) on that work were: to check the range of the site's occupation; to seek for refuse deposits, especially of long duration or in association with well known Maya and Toltec styles of architecture; to discover and record the full range and context of ceramics pertaining to the epoch of Toltec influence in Chichen Itza.

The range of the site's occupation is marked by a small collection of formative sherds from one cut, whereas in all other cuts either Puuc or Toltec-Chichen types were present, although rarely in stratigraphic sequence. As for post-Toltec-Chichen pottery the situation remains the same as described in the above-cited report.

The second objective, a search for refuse deposits, was only partly successful. Only one deposit of long duration was encountered. Located behind the Temple of the Three Lintels, it disclosed nothing later than Puuc types below the floor and mostly Toltec-Chichen pottery above. The association of ceramic deposits with either Maya or Toltec styles of architecture is still inconclusive, primarily because no strictly architectural cuts were attempted.

The third objective, involving the range and context of the Toltec-Chichen Ceramic Period, has been successfully attained.



There is nothing important to add to the summary of that period given in Year Book 53 (pp. 287-288), although a very

considerable amount of such material was encountered in the 9 cuts examined this season.

## STUDIES IN MAYA RELIGION AND HIEROGLYPHS

*J. E. S. Thompson*

Thompson spent three weeks of March in Merida, examining the effigy incense burners recovered from Mayapan during the excavations. A few are complete, but most are fragmentary. The purpose of the study was to gather information on the religious cults of Mayapan and their bearing on late Maya history.

The incense burners are large pottery vessels, often as much as 60 cm high; to the front is attached the upright figure of a god. Parts of the figures, such as feet, arms, and faces, were made in molds, details and differentiating characteristics being added in appliqué, and the parts joined with clay in a sort of primitive assembly-line technique. From ethnological sources it is known that effigy incense burners portray specific members of the Maya pantheon.

After fragments too small to be identified as specific deities, and those that lacked specific characters, had been discarded, a total of sixty-five faces suitable for study remained. Their examination led to the identification of three Mexican deities in addition to Xipe, god of human sacrifice and of agriculture, recognized by Thompson in 1954. The first is Tlazolteotl, a mother goddess, whose original home may have been Veracruz, but whose cult was widespread among the peoples of the Mexican plateau. Tlazolteotl so closely parallels the Maya goddess Ixchel-Acna in functions that the fact that her cult was able to flourish in Mayapan is of considerable interest. It may suggest a deliberate effort to keep alive practices that separated the rulers of Mayapan, with their claims to Mexican ancestry, from the rank and file of the subjected peoples.

Another Mexican deity represented on a Mayapan censer is Tlauizcalpantecutli, "lord of the dawn," an aspect of the Venus

god of the planet Venus. Finally, there is a god of merchants with a straight "Pinocchio" nose, who probably originated in southern Mexico. None of these Mexican gods is common, and the impression persists that they may have been worshipped by the relatively small group that comprised the ruling class at Mayapan.

On the other hand, Chac, the Maya rain god, who is depicted with great frequency in the Maya codices and at other Maya sites, appears somewhat infrequently on the effigy incense burners; the relative scarcity of this favorite deity of the Maya peasant in urban Mayapan, which was more interested in the political domination of the country than in farming, may be significant.

Thompson also visited the museums of Campeche and Villahermosa in search of comparative material.

In continuing work on his catalogue of Maya hieroglyphs, Thompson has carried out research on hieroglyphs for disease in general and for particular diseases. He has identified parallel passages in two of the Maya codices as divinatory almanacs for various diseases, with a glyph for each complaint written in each compartment. Thompson made a popular presentation of his findings at the annual meeting of the Society for American Archaeology in Madison this past May in an endeavor to acquaint colleagues in the general field of anthropology with the nature of the problems of Maya hieroglyphic decipherment and how they are being attacked here.

Thompson also carried out research on the use of cacao in the religious and economic life of ancient Middle America and on fluctuations in its value as a currency in the colonial period. Results have appeared in a paper published by this Department (see below).

## PUBLICATIONS

H. E. D. Pollock

One book, mentioned in our last report as a manuscript submitted for publication, has recently appeared. *The Political Geography of the Yucatan Maya* (Publication 613), by Ralph L. Roys, was published in June 1957. This addition to our series of historical studies was composed on IBM typewriter and printed by offset. The book is an investigation of the sixteen independent Maya states of Yucatan at the time of the Spanish conquest in 1542. The boundaries and most of the towns of these states are mapped and described, and a study is made of economic and political conditions wherever possible.

Four papers have been added to the fifth volume of Notes on Middle American Archaeology and Ethnology: *Chronological Decipherments from Uaxactun, Naranjo, and Ixlu, Peten* (no. 127), by J. Eric S. Thompson; *Notes on the Use of Cacao in Middle America* (no. 128), by J. Eric S. Thompson; *Tohil Plumbate and Classic Maya Polychrome Vessels in the Márquez Collection* (no. 129), by Robert E. Smith;

*A New Inscription from the Temple of the Foliated Cross at Palenque* (no. 130), by Heinrich Berlin.

The second volume of Current Reports has continued with the addition of seven papers: *A Dwelling and Shrine at Mayapan* (no. 33), by Ann Chowning and Donald E. Thompson; *A Round Temple and Its Shrine at Mayapan* (no. 34), by Ann Chowning; *Exploration of the Cave of Dzab-Na, Tecoh, Yucatan* (no. 35), by Gustav Strömsvik; *Excavations in House Mounds at Mayapan: IV* (no. 36), by A. Ledyard Smith and Karl Ruppert; *The Southern Terminus of the Principal Sacbe at Mayapan—Group Z-50* (no. 37), by H. E. D. Pollock; *Skeletal Remains from Mayapan, Yucatan* (no. 38), by Edward I. Fry; *House Types in the Environs of Mayapan and at Uxmal, Kabah, Sayil, Chichen Itza, and Chacchob* (no. 39), by Karl Ruppert and A. L. Smith.

One more paper in the Notes series and two Current Reports are in press. They should appear in the autumn of 1957.

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## PERSONNEL

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| Anna O. Shepard                                      | * Retired October 1, 1956. |
| Edwin M. Shook ‡                                     | ‡ Absent on leave.         |
|  | § Retired July 1, 1957.    |

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*Year Book 55, 1955-1956.* Octavo, xi + 343 + XXX pages, 1 plate, 110 figures, 1956.

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*Caryl P. Haskins*

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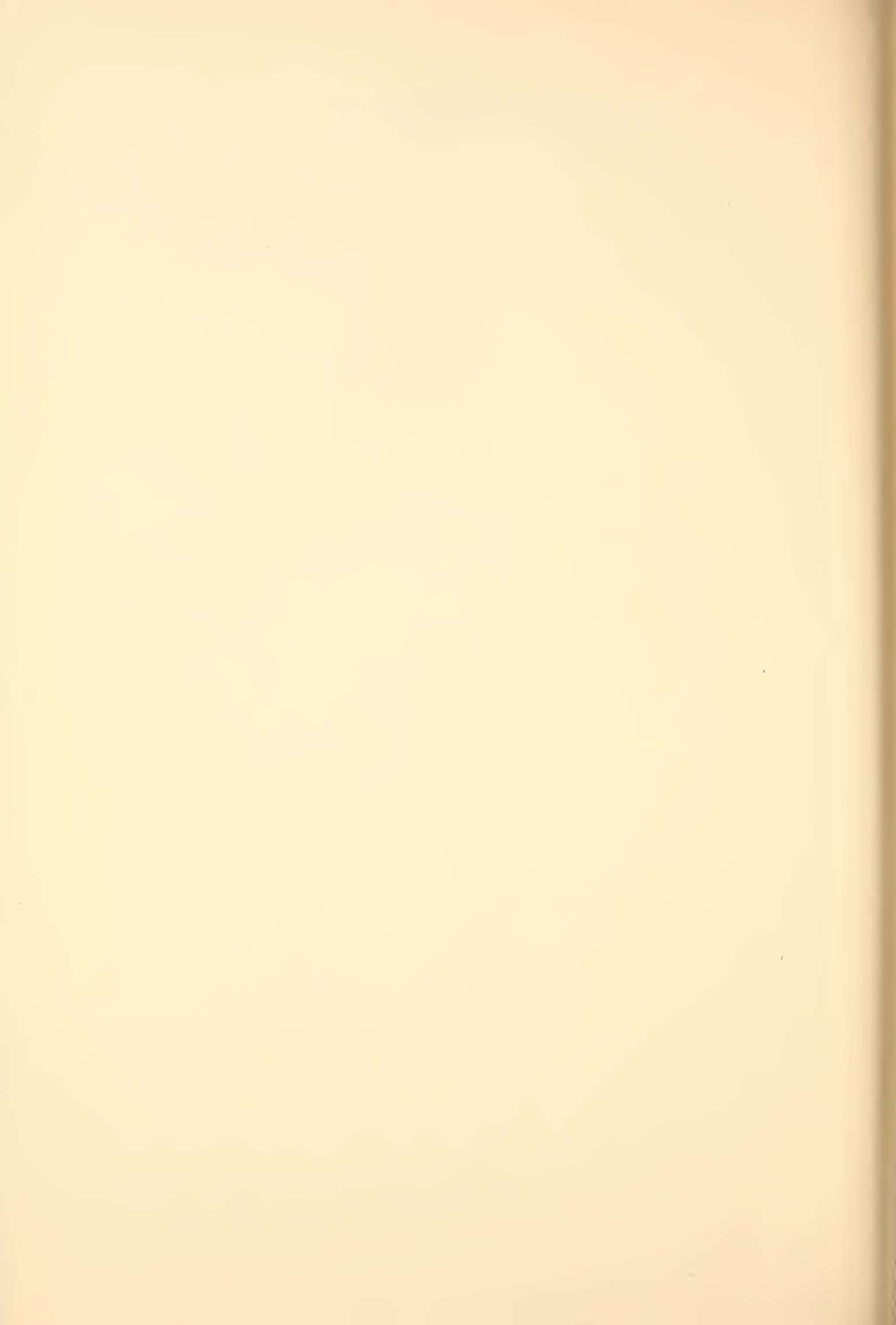




# SUPPLEMENT

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## REPORT OF THE EXECUTIVE COMMITTEE

For the Year Ended June 30, 1957

*To the Trustees of the Carnegie Institution of Washington:*

*Gentlemen:* In accordance with the provisions of the By-Laws, the Executive Committee submits this report to the annual meeting of the Board of Trustees.

During the fiscal year ended June 30, 1957, the Executive Committee held three meetings, printed reports of which have been mailed to each Trustee and constitute a part of this report.

The report of the President has been received by the Executive Committee and is presented with its approval. The Executive Committee has reviewed and approves the proposed appropriation for the operation of the Institution from January 1, 1958, through June 30, 1958.

One vacancy exists in the membership of the Board of Trustees.

The following Officers and Committee Members were elected (or appointed) for terms ending in 1957:

### *Officers of Board of Trustees*

Walter S. Gifford, Chairman

Barklie McKee Henry, Vice-Chairman

Robert Woods Bliss, Secretary

#### *Executive Committee*

Henry S. Morgan

Henry R. Shepley

#### *Finance Committee*

Alfred L. Loomis

Henry S. Morgan

Walter S. Gifford

#### *Auditing Committee*

Alfred L. Loomis

#### *Retirement Committee*

Barklie McKee Henry

#### *Nominating Committee*

Crawford H. Greenewalt

Barklie McKee Henry, Chairman of Executive Committee

Lindsay Bradford, Chairman of Finance Committee

Keith S. McHugh, Chairman of Auditing Committee

Lindsay Bradford, Chairman of Retirement Committee

Elihu Root, Jr., Chairman of Nominating Committee

*Committee on Astronomy:* Seeley G. Mudd, Chairman, Crawford H. Greenewalt, Elihu Root, Jr.

*Committee on Terrestrial Sciences:* Ernest O. Lawrence, Chairman, Barklie McKee Henry, Henning W. Prentis, Jr., Robert E. Wilson.

*Committee on Biological Sciences:* Alfred L. Loomis, Chairman, Margaret Carnegie Miller, William I. Myers, Charles P. Taft.

*Committee on Archaeology:* Henry R. Shepley, Chairman, James F. Bell, Robert Woods Bliss, Juan T. Trippe.

BARKLIE MCKEE HENRY, *Chairman*

October 24, 1957





HASKINS & SELLS  
*Certified Public Accountants*

*First National Bank Building*  
*Baltimore 2*

#### ACCOUNTANTS' CERTIFICATE

*To the Auditing Committee of Carnegie Institution of Washington:*

We have examined the balance sheet of Carnegie Institution of Washington as of June 30, 1957 and the related summaries of current income and expenditures, current funds surplus, current restricted gifts and grants, changes in endowment and other special funds, and changes in investment in real estate and equipment for the year then ended (Exhibits A to F, inclusive). Our examination was made in accordance with generally accepted auditing standards, and accordingly included such tests of the accounting records and such other auditing procedures as we considered necessary in the circumstances.

In our opinion, the accompanying balance sheet and above described summaries (Exhibits A to F, inclusive) present fairly the financial position of the Institution at June 30, 1957 and the results of its operations for the year then ended, in conformity with generally accepted accounting principles applied on a basis consistent with that of the preceding year.

*August 20, 1957*

HASKINS & SELLS



## ASSETS

Current Funds:

Cash .....		\$465,958.10	
Advances:			
Departmental Research Operations .....		13,566.81	
Other .....		1,839.55	
Accounts receivable .....		633.20	
Deferred charges .....		35,789.80	
Due from Endowment and Other Special Funds .....		325,072.59	\$842,860.05

Endowment and Other Special Funds:

Cash .....		\$51,850.00	
Securities (valuation based on market quotations at June 30, 1957—\$73,248,809)—Schedule 1:			
Bonds .....	\$38,241,589.26		
Preferred stocks .....	3,431,844.40		
Common stocks .....	13,957,652.39	55,631,086.05	55,682,936.05

Plant Funds:

Investment in real estate and equipment—Exhibit F .....			5,375,540.67
Total .....			\$61,901,336.77

## LIABILITIES

Current Funds:

Accounts payable .....		\$4,519.68	
Reserve for accounts receivable .....		633.20	
Current Funds Surplus—Exhibit C:			
Appropriated unexpended balances .....	\$592,602.95		
General Contingent Fund .....	110,513.04	703,115.99	
Unexpended balance of restricted gifts and grants—Exhibit D .....		134,591.18	\$842,860.05

Endowment and Other Special Funds:

Due to Current Funds .....		\$325,072.59	
Principal of Funds—Exhibit E:			
Capital funds .....	\$51,732,053.69		
Special funds .....	3,625,809.77	55,357,863.46	55,682,936.05

Plant Funds:

Bequests, gifts, and income invested in plant .....		\$5,332,040.67	
Harriman Fund—donated land .....		5,500.00	
Hale Fund—Solar Laboratory .....		38,000.00	5,375,540.67
Total .....			\$61,901,336.77

SUMMARY OF CURRENT INCOME AND EXPENDITURES  
FOR THE YEAR ENDED JUNE 30, 1957

Current Income:

## Investment income:

Interest and dividends on securities .....	\$2,357,254.22	
Less: Amortization of bond premiums .....	24,062.57	\$2,333,191.65

Market value of stock dividend .....		7,210.88
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Total—Schedule 1 .....		\$2,340,402.53
Less: Income added to Special Funds (Exhibit E)—Schedule 1 .....		5,079.27

Remainder—Income available for current purposes .....		\$2,335,323.26
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## Other income:

Sales of publications .....	\$6,456.03	
Dormitory and mess hall .....	10,859.71	
Miscellaneous .....	5,367.77	22,683.51

Restricted gifts and grants utilized for current purposes—Exhibit D .....		91,165.91
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Total Current Income .....		\$2,449,172.68
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Current Expenditures (including expenditures against appropriations  
of prior years)—Schedule 4:

Administration .....	\$327,382.41	
Departmental Research Operations .....	1,372,653.17	
General Publications .....	29,344.92	
Research Projects, Fellowships, Grants, etc. ....	171,606.83	
Hospitalization Plan .....	15,939.92	
Pension Fund—annuity and insurance .....	95,138.38	
Retirement Plan Contributions .....	177,760.08	

\$2,189,825.71

Gifts and grants—Exhibit D .....	91,165.91	
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Total Current Expenditures .....		2,280,991.62
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Excess of Current Income over Current Expenditures—Exhibit C .....		\$168,181.06 *
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## \* Summarized as follows:

Current investment income in excess of Trustees' authorized  
appropriations during the fiscal year, credited to General

Reserve Fund—Exhibit E .....	\$264,192.53	
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Transfer of unexpended current appropriations to General Contingent

Fund—Schedule 4 .....	82,474.08	
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Reserved from this year's appropriations for current liabilities and  
commitments—Schedule 4 .....

188,601.66

Total .....	\$535,268.27	
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Less: Amount included in current expenditures, applicable to  
allotments and unexpended balances from prior years'

appropriations—Schedule 4 .....	367,087.21	\$168,181.06
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## EXHIBIT C

SUMMARY OF CURRENT FUNDS SURPLUS  
FOR THE YEAR ENDED JUNE 30, 1957

Balance, July 1, 1956 .....				\$700,711.04
<u>Additions:</u>				
Excess of current income over current expenditures—Exhibit B .....		\$168,181.06		
Transfers from Special Funds—Exhibit E:				
Special Purpose Funds .....	\$5,000.00			
General Reserve Fund .....	100,000.00	105,000.00		273,181.06
				<hr/>
Total .....				\$973,892.10
 <u>Deductions:</u>				
Transfers to Special Funds—Exhibit E:				
General Reserve Fund, representing excess of investment				
income over Trustees' appropriations for current				
purposes .....		\$264,192.53		
Special Purpose Funds .....		650.25		
Harriet H. Mayor Relief Fund .....		5,933.33		270,776.11
				<hr/>
Balance, June 30, 1957, per Schedule 3 .....				\$703,115.99
				<hr/> <hr/>

EXHIBIT D

SUMMARY OF CURRENT RESTRICTED GIFTS AND GRANTS  
FOR THE YEAR ENDED JUNE 30, 1957

	Unexpended Balance June 30, 1956	Additions— Gifts and Grants Received	Deductions— Expenditures (Schedule 4)	Unexpended Balance June 30, 1957
<u>Departmental Research Operations:</u>				
Department of Genetics:				
American Cancer Society No. EG 21 .....	\$2,832.65	\$8,500.00	\$6,322.41	\$5,010.24
U. S. Public Health Service No. RG-149 C ..	2,722.59	9,637.00	9,364.15	2,995.44
U. S. Public Health Service No. C-2158 C ..	2,252.06	12,000.00	11,144.08	3,107.98
Department of Terrestrial Magnetism:				
National Science Foundation No. 1196-1 .....	11,516.60	.....	6,757.10	4,759.50
National Science Foundation No. 2236 .....	2,479.68	.....	2,479.68	...
National Science Foundation No. 2699 .....	.....	5,000.00	273.17	4,726.83
National Science Foundation No. y/3.16/169 ..	.....	22,500.00	2,792.05	19,707.95
National Science Foundation No. y/11.11/170. ....	.....	34,050.00	12,849.72	21,200.28
National Science Foundation No. 3549 .....	.....	9,000.00	.....	9,000.00
Department of Embryology:				
U. S. Public Health Service - Greenwald ...	30.21	.....	30.21	.....
U. S. Public Health Service - De Feo .....	.....	500.00	500.00	.....
Population Council Inc. ....	9,350.00	.....	6,838.81	2,511.19
Mount Wilson Observatory:				
Gift .....	2,000.00	1,500.00	.....	3,500.00
<u>Research Projects, Fellowships, etc.:</u>				
Carnegie Corporation of New York:				
Biology:				
Yerkes Laboratories of Primate Biology .....	.....	10,000.00	10,000.00	.....
Geology:				
Tilley, C. E. ....	620.63	.....	.....	620.63
Natural Sciences:				
Fellowships .....	.....	50,000.00	15,000.00	35,000.00
Physiology:				
Russell, G. Oscar .....	1,222.44	.....	.....	1,222.44
Terrestrial Magnetism:				
Clews, C. J. Birkett .....	158.64	.....	158.64	.....
Telescope image converter .....	22,884.59	.....	1,655.89	21,228.70
Tuve, M. A., travel .....	5,000.00	.....	5,000.00	.....
Total .....	\$63,070.09	\$162,687.00	\$91,165.91	\$134,591.18



EXHIBIT E SUMMARY OF CHANGES IN ENDOWMENT AND OTHER SPECIAL FUNDS FOR THE YEAR ENDED JUNE 30, 1957

		Additions			Deductions		
		Gifts, Sale of Property, Transfers, and Misc.	Income from Investments Added to Funds (Exhibit B)	Net Realized Gain on Investments (Schedule 2)	Transfers to Current Funds Surplus (Exhibit C)	Expendi- tures	Balance June 30, 1957
<u>Capital Funds:</u>							
Endowment Fund .....	\$32,000,000.00	.....	.....	.....	.....	.....	\$32,000,000.00
Capital Reserve Fund .....	17,223,825.77	\$139,474.70	.....	\$2,248,224.37	.....	.....	19,611,524.84
Colburn Fund .....	103,310.80	.....	.....	.....	.....	.....	103,310.80
Harkavy Fund .....	5,051.05	.....	.....	.....	.....	.....	5,051.05
Teeples Fund .....	10,888.42	.....	.....	.....	.....	.....	10,888.42
Van Gelder Fund .....	1,278.58	.....	.....	.....	.....	.....	1,278.58
<u>Special Funds:</u>							
Bickel Fund .....	12,089.29	.....	\$514.19	.....	.....	.....	12,603.48
General Reserve Fund .....	3,243,768.62	93,024.70	264,192.53	.....	\$100,000.00	.....	3,500,985.85
George E. Hale Relief Fund .....	4,537.22	.....	152.57	.....	.....	.....	4,689.79
Harkavy Fund—Income.....	1,272.74	.....	268.97	.....	.....	.....	1,541.71
Harriet H. Mayor Relief Fund .....	4,516.67	5,933.33	.....	.....	.....	\$2,350.00	8,100.00
Special Income Reserve .....	228,571.89	— 228,571.89 *	.....	.....	.....	.....	...
Special Purpose Funds .....	75,880.79	2,274.25	3,267.78	.....	5,000.00	.....	76,422.82
Woloff Fund .....	20,590.36	.....	875.76	.....	.....	.....	21,466.12
Total .....	\$52,935,582.20	\$12,135.09	\$269,271.80	\$2,248,224.37	\$105,000.00	\$2,350.00	\$55,357,863.46
<u>* Transferred to Capital Reserve Fund .....</u> \$139,474.70							
<u>Transferred to General Reserve Fund .....</u> 89,097.19							
<u>Total .....</u> \$228,571.89							

EXHIBIT F SUMMARY OF CHANGES IN INVESTMENT IN REAL ESTATE AND EQUIPMENT FOR THE YEAR ENDED JUNE 30, 1957

	Classification of June 30, 1957 Balance							
	Balance July 1, 1956	Additions (see Note)	Deductions	Balance June 30, 1957	Buildings and Grounds	Laboratory Apparatus	Library	Operating Equipment
Departments of Research:								
Department of Plant Biology Stanford, California .....	\$165,237.22	\$8,381.59	\$915.97	\$172,702.84	\$75,519.58	\$50,949.28	\$28,633.83	\$17,600.15
Department of Genetics Long Island, New York .....	1,177,573.97	6,308.06	4,296.90	1,179,585.13	984,150.41	81,874.12	78,872.58	34,688.02
Geophysical Laboratory Washington, D. C. ....	488,684.36	8,128.13	785.40	496,027.09	170,383.79	213,427.18	49,926.07	62,290.05
Department of Archaeology Cambridge, Massachusetts .....	12,252.76	.....	484.56	11,768.20	.....	.....	1,154.67	10,613.53
Mount Wilson Observatory Pasadena, California .....	1,671,058.55	62,265.52	33,213.06	1,700,111.01	275,827.70	1,266,911.03	83,404.00	73,968.28
Department of Terrestrial Magnetism Washington, D. C. ....	837,811.88	20,443.61	3,973.69	854,281.80	401,418.69	325,832.34	48,862.14	78,168.63
Department of Embryology Baltimore, Maryland .....	80,232.28	24,231.37	179.93	104,283.72	.....	86,229.10	10,006.56	8,048.06
Total Departments of Research ..	\$4,432,851.02	\$129,758.28	\$43,849.51	\$4,518,759.79	\$1,907,300.17	\$2,025,223.05	\$300,859.85	\$285,376.72
Office of Administration								
Washington, D. C.	843,160.23	13,752.81	132.16	856,780.88	811,385.77	.....	.....	\$ 45,395.11
Total .....	\$5,276,011.25	\$143,511.09	\$43,981.67	\$5,375,540.67	\$2,718,685.94	\$2,025,223.05	\$300,859.85	\$330,771.83
Note: Additions during year provided from following:								
Current expenditures for equipment—Schedule 4 .....								
Cost of constructed equipment capitalized:								
Mount Wilson Observatory .....								
Total, as above .....								



SCHEDULE 1                      ENDOWMENT AND OTHER SPECIAL FUNDS INVESTMENTS AS OF  
JUNE 30, 1957 AND INCOME THEREFROM DURING THE YEAR

	Book Value	Market Value	Per Cent of Total Investments		Income Received
			Book Value	Market Value	
Bonds:					
United States Government .....	\$10,426,122.96	\$10,090,412	18.72%	13.77%	\$339,725.99
Foreign and International Bank .....	2,274,987.45	2,159,750	4.09%	2.95%	37,665.21
Public Utility .....	7,013,445.84	6,236,152	12.60%	8.51%	167,602.23
Communication .....	3,871,763.16	3,488,750	6.95%	4.76%	64,718.22
Railroad .....	473,342.86	448,890	0.85%	0.61%	20,588.95
Railroad Equipment Trust .....	780,864.60	756,205	1.40%	1.03%	20,632.49
Industrial and Miscellaneous .....	13,401,062.39	12,402,386	24.07%	16.92%	355,129.21
Total bonds .....	<u>\$38,241,589.26</u>	<u>\$35,582,545</u>	<u>68.68%</u>	<u>48.55%</u>	<u>\$1,006,062.30 *</u>
Stocks:					
Preferred .....	\$3,431,844.40	\$2,896,922	6.16%	3.95%	\$150,028.84
Common .....	13,957,652.39	34,769,342	25.07%	47.43%	1,184,311.39 †
Total stocks .....	<u>\$17,389,496.79</u>	<u>\$37,666,264</u>	<u>31.23%</u>	<u>51.38%</u>	<u>\$1,334,340.23</u>
Cash .....	<u>\$51,850.00</u>	<u>\$51,850</u>	<u>0.09%</u>	<u>0.07%</u>	<u>.....</u>
Total .....	<u>\$55,682,936.05</u>	<u>\$73,300,659</u>	<u>100.00%</u>	<u>100.00%</u>	<u>\$2,340,402.53 ‡</u>

\* After deducting bond premium amortization of \$24,062.57.

† Includes \$7,210.88 representing market value of a stock dividend received.

‡ Income received allocated to Endowment and Other Special Funds as follows:

Funds, the income from which may be used for current general purposes—Exhibit B .....	\$2,335,323.26
Funds, the income from which is restricted to specific purposes—Exhibit E:	
Bickel Fund .....	\$514.19
George E. Hale Relief Fund .....	152.57
Harkavy Fund .....	268.97
Special Purpose Funds .....	3,267.78
Woloff Fund .....	875.76
Total .....	<u>5,079.27</u>
	<u><u>\$2,340,402.53</u></u>

## SCHEDULE 2

## SCHEDULE OF SECURITIES

Principal Amount	Description	Maturity	Book Value	Approximate Market Value
United States Government Bonds				
\$600,000	U. S. of America Treasury Notes Series "C" 2s .....	1957	\$597,468.76	\$598,875
3,600,000	U. S. of America Treasury Notes Series "D" 2 $\frac{3}{4}$ s .....	1957	3,589,281.28	3,597,750
1,105,000	U. S. of America Treasury 2 $\frac{1}{4}$ s .....	1962-59	1,110,645.48 *	1,022,125
1,500,000	U. S. of America Treasury 2 $\frac{1}{2}$ s .....	1958	1,498,089.77	1,474,219
300,000	U. S. of America Treasury 2 $\frac{1}{2}$ s .....	1961	295,241.84	282,000
2,233,000	U. S. of America Treasury 2 $\frac{1}{2}$ s .....	1963	2,235,395.83 *	2,059,943
800,000	U. S. of America Treasury 2 $\frac{3}{4}$ s .....	1961	800,000.00	762,000
100,000	U. S. of America Savings Series "G" 2 $\frac{1}{2}$ s .....	1958	100,000.00	98,600
100,000	U. S. of America Savings Series "G" 2 $\frac{1}{2}$ s .....	1959	100,000.00	97,900
100,000	U. S. of America Savings Series "G" 2 $\frac{1}{2}$ s .....	1960	100,000.00	97,000
<u>\$10,438,000</u>	<u>Total U. S. Government .....</u>		<u>\$10,426,122.96</u>	<u>\$10,090,412</u>
Foreign and International Bank Bonds				
\$250,000	Aluminum Company of Canada, Ltd., S. F. Deb. 3 $\frac{7}{8}$ s Guar. ...	1970	\$252,672.12 *	\$243,750
500,000	Aluminum Company of Canada, Ltd., S. F. Deb. 4 $\frac{1}{2}$ s .....	1980	511,252.50	505,000
150,000	Australia, Commonwealth of, 4 $\frac{1}{2}$ s .....	1971	147,750.00	144,375
150,000	Australia, Commonwealth of, 5s .....	1972	150,000.00	150,375
250,000	British Columbia Power Commission, S. F. Deb. Series "L" 4 $\frac{3}{8}$ s .....	1987	245,000.00	239,375
100,000	Canadian National Ry. Co., 4 $\frac{1}{2}$ s Guar. ....	1957	112,000.00	100,000
125,000	International Bank for Reconstruction and Development, 3s .	1976	125,000.00	102,500
125,000	International Bank for Reconstruction and Development, 3 $\frac{3}{8}$ s	1975	123,125.00	109,375
250,000	International Bank for Reconstruction and Development, 4 $\frac{1}{2}$ s	1977	250,000.00	250,000
150,000	Noranda Mines Ltd., S. F. Deb. 4 $\frac{3}{4}$ s .....	1968	153,147.83 *	147,000
200,000	Shawinigan Water & Power Co., 1st Mtg. & Coll. Tr. S. F. Series "M" 3s .....	1971	205,040.00 *	168,000
<u>\$2,250,000</u>	<u>Total Foreign and International Bank .....</u>		<u>\$2,274,987.45</u>	<u>\$2,159,750</u>
Public Utility Bonds				
\$250,000	California Oregon Power Co., 1st Mtg. 3 $\frac{7}{8}$ s .....	1986	\$253,218.34 *	\$215,000
125,000	Columbia Gas System, Inc., Series "B" 3s .....	1975	127,443.53 *	102,500
250,000	Columbia Gas System, Inc., Series "F" 3 $\frac{7}{8}$ s .....	1981	245,937.50	230,000
237,000	Columbus & Southern Ohio Electric Co., 1st Mtg. 3 $\frac{1}{4}$ s .....	1970	245,462.72 *	208,560
300,000	Commonwealth Edison Company, 1st Mtg. Series "R" 3 $\frac{1}{2}$ s ..	1986	300,758.34 *	274,500
300,000	Consolidated Edison Co. of N. Y., 1st & Ref. Mtg. Series "L" 3 $\frac{5}{8}$ s .....	1986	303,990.31 *	273,000
300,000	Consolidated Natural Gas Co., Deb. 2 $\frac{3}{4}$ s .....	1968	300,462.52 *	271,500
150,000	Consumers Power Company, 1st Mtg. 4s .....	1986	151,472.22 *	144,750
300,000	Florida Power Corporation, 1st Mtg. 3 $\frac{7}{8}$ s .....	1986	302,215.15 *	270,000
500,000	Illinois Power Company, 1st Mtg. 3 $\frac{1}{4}$ s .....	1986	497,937.50	450,000
200,000	Minnesota Power & Light Co., 1st Mtg. 3 $\frac{1}{8}$ s .....	1975	202,977.57 *	160,000
250,000	Niagara Mohawk Power Corp., Gen. Mtg. 3 $\frac{5}{8}$ s .....	1986	253,319.50 *	223,750
100,000	Ohio Power Co., 1st Mtg. 3 $\frac{1}{4}$ s .....	1968	101,500.00	89,250
200,000	Pacific Gas and Electric Co., 1st & Ref. Mtg. Series "X" 3 $\frac{1}{8}$ s	1984	201,574.98 *	160,000
300,000	Pacific Gas and Electric Co., 1st & Ref. Mtg. Series "Y" 3 $\frac{3}{8}$ s	1987	306,724.59 *	255,000
200,000	Panhandle Eastern Pipe Line Co., Serial Deb. 2 $\frac{3}{4}$ s .....	1961-62	200,855.11 *	183,750
87,000	Panhandle Eastern Pipe Line Co., S. F. Deb. 3 $\frac{1}{4}$ s .....	1973	88,000.66 *	78,300
50,000	Philadelphia Electric Co., 1st & Ref. Mtg. 2 $\frac{1}{2}$ s .....	1978	49,687.50	41,000
207,000	Philadelphia Electric Power Co., 1st Mtg. 2 $\frac{3}{8}$ s Guar. ....	1975	210,131.12 *	178,020
250,000	Potomac Electric Power Co., Deb. 4 $\frac{5}{8}$ s .....	1982	256,550.00	248,125
200,000	Public Service Co. of Indiana, 1st Mtg. Series "F" 3 $\frac{1}{8}$ s .....	1975	203,042.51 *	160,000
250,000	Southern California Edison Co., 1st & Ref. Mtg. Series "G" 3 $\frac{5}{8}$ s .....	1981	247,765.00	225,000
250,000	Southern California Edison Co., 1st & Ref. Mtg. Series "H" 4 $\frac{1}{4}$ s .....	1982	251,875.00	243,125
210,000	Tennessee Gas Transmission Co., 1st Mtg. Pipe Line 2 $\frac{3}{4}$ s ...	1966	211,417.50 *	174,300
191,000	Tennessee Gas Transmission Co., 1st Mtg. Pipe Line 3s ....	1969	194,402.98 *	160,440
300,000	Tennessee Gas Transmission Co., 1st Mtg. Pipe Line 5 $\frac{1}{4}$ s ...	1977	300,000.00	307,500
500,000	Union Electric Company, 1st Mtg. 3 $\frac{3}{4}$ s .....	1986	500,110.75 *	455,000
265,000	United Gas Corp., 1st Mtg. & Coll. Tr. 2 $\frac{3}{4}$ s .....	1967	265,000.00	232,882
235,000	Virginia Electric & Power Co., 1st & Ref. Mtg. Series "M" 4 $\frac{1}{2}$ s .....	1986	239,612.94 *	220,900
<u>\$6,957,000</u>	<u>Total Public Utility .....</u>		<u>\$7,013,445.84</u>	<u>\$6,236,152</u>

\*After deduction for amortization of premiums on bonds purchased subsequent to January 1, 1940.



SCHEDULE OF SECURITIES—Continued

Principal Amount	Description	Maturity	Book Value	Approximate Market Value
Communication Bonds				
\$150,000	American Telephone & Telegraph Co., Deb. 2 $\frac{3}{4}$ s .....	1975	\$151,518.75 *	\$119,625
350,000	American Telephone & Telegraph Co., Deb. 3 $\frac{1}{4}$ s .....	1984	361,524.68 *	293,125
800,000	American Telephone & Telegraph Co., Deb. 3 $\frac{7}{8}$ s .....	1990	821,620.68 *	728,000
500,000	American Telephone & Telegraph Co., Deb. 4 $\frac{1}{8}$ s .....	1985	506,070.00	486,250
400,000	Illinois Bell Telephone Co., 1st Mtg. Series "E" 4 $\frac{1}{4}$ s .....	1988	405,536.00	395,000
200,000	Mountain States Telephone & Telegraph Co., Deb. 3 $\frac{1}{8}$ s .....	1978	201,120.00 *	152,000
100,000	New York Telephone Co., Ref. Mtg. Series "E" 3 $\frac{1}{8}$ s .....	1978	100,986.19 *	86,000
200,000	Pacific Telephone & Telegraph Co., Deb. 3 $\frac{1}{4}$ s .....	1978	203,531.74 *	176,000
300,000	Pacific Telephone & Telegraph Co., Deb. 4 $\frac{1}{8}$ s .....	1988	307,444.02 *	297,000
250,000	Southern Bell Telephone & Telegraph Co., Deb. 4s .....	1983	251,361.10 *	240,000
250,000	Southern Bell Telephone & Telegraph Co., Deb. 5s .....	1986	255,800.00	263,750
300,000	Southwestern Bell Telephone Co., Deb. 3 $\frac{1}{8}$ s .....	1983	305,250.00 *	252,000
<u>\$3,800,000</u>	<u>Total Communication .....</u>		<u>\$3,871,763.16</u>	<u>\$3,488,750</u>
Railroad Bonds				
\$100,000	Chesapeake & Ohio Ry. Co., Gen. Mtg. 4 $\frac{1}{2}$ s .....	1992	\$99,464.29	\$103,500
267,000	Fort Worth & Denver Rwy. Co., 1st Mtg. 4 $\frac{3}{8}$ s Guar. ....	1982	269,216.07 *	245,640
100,000	Pennsylvania R. R. Co., Cons.Mtg. 4 $\frac{1}{2}$ s .....	1960	104,662.50	99,750
<u>\$467,000</u>	<u>Total Railroad .....</u>		<u>\$473,342.86</u>	<u>\$448,890</u>
Railroad Equipment Trust Bonds				
\$50,000	Chesapeake & Ohio Ry. Co., Eq. Tr. 2s Guar. ....	1958	\$48,454.40	\$49,370
300,000	Chicago Burlington & Quincy R. R. Co., Eq. Tr. 2 $\frac{1}{4}$ s Guar. ..	1958-63	292,507.12	279,630
100,000	Great Northern Railway Co., Eq. Tr. 2s Guar. ....	1960-61	98,538.91	91,880
150,000	Pennsylvania R. R. Co., Eq. Tr. Series "S" 2 $\frac{3}{8}$ s Guar. ....	1958-62	146,358.96	139,935
50,000	Southern Pacific Co., Eq. Tr. Series "CC" 2 $\frac{1}{8}$ s Guar. ....	1959	49,665.94	47,185
50,000	Southern Pacific Co., Eq. Tr. Series "X" 2 $\frac{1}{8}$ s Guar. ....	1958	48,359.01	48,880
100,000	Southern Railway Co., Eq. Tr. Series "NN" 2 $\frac{1}{8}$ s Guar. ....	1957-58	96,980.26	99,325
<u>\$800,000</u>	<u>Total Railroad Equipment Trust .....</u>		<u>\$780,864.60</u>	<u>\$756,205</u>
Industrial and Miscellaneous Bonds				
\$200,000	Allied Chemical and Dye Corp., Deb. 3 $\frac{1}{2}$ s .....	1978	\$198,000.00	\$183,250
200,000	Aluminum Company of America, S. F. Deb. 3 $\frac{1}{8}$ s .....	1964	200,000.00	193,750
100,000	Aluminum Company of America, S. F. Deb. 3s .....	1979	100,000.00	85,000
250,000	Aluminum Company of America, S. F. Deb. 4 $\frac{1}{4}$ s .....	1982	250,000.00	255,000
187,000	American Tobacco Co., Deb. 3s .....	1969	188,619.23 *	170,638
234,000	Bristol-Myers Co., Deb. 3s .....	1968	234,621.26 *	214,110
500,000	Burroughs Corporation, Conv. Sub. Deb. 4 $\frac{1}{2}$ s .....	1981	540,450.92 *	585,000
300,000	C. I. T. Financial Corp., Deb. 2 $\frac{5}{8}$ s .....	1959	300,000.00 *	288,375
125,000	Carrier Corporation, Conv. Sub. Deb. 4 $\frac{1}{8}$ s .....	1982	126,875.00	123,750
400,000	Commercial Credit Co., Notes 3 $\frac{5}{8}$ s .....	1976	409,387.76 *	356,000
400,000	Continental Oil Company, S. F. Deb. 3s .....	1984	404,569.00 *	344,000
500,000	Crown Zellerbach Corporation, Prom. Note 4 $\frac{1}{8}$ s .....	1981	500,000.00	455,100
150,000	Dow Chemical Co., Deb. 2.35s .....	1961	150,238.65 *	138,000
130,000	Dow Chemical Co., Conv. Sub. Deb. 3s .....	1982	131,662.12 *	181,350
153,000	Food Machinery Corp., S. F. Deb. 2 $\frac{1}{2}$ s .....	1962	152,308.98	140,760
500,000	Food Machinery and Chemical Corporation, S. F. Deb. 3.80s	1981	500,000.00	485,000
300,000	General American Transportation Corporation, Conv. Sub. Deb. 4s .....	1981	336,416.22 *	325,875
500,000	General Electric Co., Deb. 3 $\frac{1}{2}$ s .....	1976	502,368.48 *	465,625
250,000	General Motors Acceptance Corp., Deb. 3s .....	1960	250,000.00	237,500
200,000	General Motors Acceptance Corp., Deb. 3 $\frac{1}{2}$ s .....	1972	204,500.02 *	173,750
180,000	General Motors Acceptance Corp., Deb. 4s .....	1958	180,000.00	179,550
500,000	General Motors Corporation, Deb. 3 $\frac{1}{4}$ s .....	1979	502,125.00 *	442,500
275,000	Goodrich (B. F.) Company, 1st Mtg. 2 $\frac{3}{4}$ s .....	1965	275,506.65 *	247,500
500,000	Illinois State Toll Highway Comm., Rev. Bonds 3 $\frac{3}{4}$ s .....	1995	500,000.00	397,500
236,000	Lorillard (P.) Co., Deb. 3s .....	1963	238,661.58 *	212,400
500,000	National Cash Register Co., Conv. Sub. Deb. 4 $\frac{1}{2}$ s .....	1981	553,504.47 *	615,000
295,000	National Dairy Products Corp., Deb. 2 $\frac{3}{4}$ s .....	1970	297,640.36 *	252,225
488,000	Phillips Petroleum Co., S. F. Deb. 2 $\frac{3}{4}$ s .....	1964	490,933.81 *	453,840
125,000	Pittsburgh Plate Glass Co., S. F. Deb. 3s .....	1967	125,000.00	116,250

\*After deduction for amortization of premiums on bonds purchased subsequent to January 1, 1940.

SCHEDULE OF SECURITIES—Continued

Principal Amount	Description	Maturity	Book Value	Approximate Market Value
<u>Industrial and Miscellaneous Bonds—Concluded</u>				
\$150,000	Quaker Oats Co., Deb. 2 $\frac{5}{8}$ s .....	1964	\$148,922.50	\$136,500
100,000	Riegel Paper Corp., S. F. Deb. 3 $\frac{7}{8}$ s .....	1981	100,000.00	90,000
250,000	Scovill Manufacturing Co., Deb. 4 $\frac{3}{4}$ s .....	1982	246,250.00	250,000
300,000	Seagram (Joseph E.) & Sons, Inc., Deb. 2 $\frac{1}{2}$ s .....	1966	298,500.00	256,500
275,000	Sears Roebuck Acceptance Corp., Sub. Deb. 4 $\frac{5}{8}$ s .....	1977	274,005.00	264,688
300,000	Service Pipe Line Co., S. F. Deb. 3.20s .....	1982	300,000.00	270,000
500,000	Shell Union Oil Corp., Deb. 2 $\frac{1}{2}$ s .....	1971	502,749.65 *	410,000
300,000	Sinclair Oil Corporation, Conv. Sub. Deb. 4 $\frac{3}{8}$ s .....	1986	317,935.36 *	327,000
300,000	Superior Oil Company, The (California), Deb. 3 $\frac{3}{4}$ s .....	1981	300,000.00	276,000
300,000	Swift & Co., Deb. 2 $\frac{5}{8}$ s .....	1972	301,051.25 *	258,000
500,000	Texas Corporation, Deb. 3s .....	1965	512,501.73 *	470,000
250,000	Tide Water Associated Oil Co., S. F. Deb. 3 $\frac{1}{2}$ s .....	1986	250,000.00	227,500
346,000	Union Oil Company of California, Deb. 2 $\frac{3}{4}$ s .....	1970	352,985.47 *	294,100
400,000	Westinghouse Electric Corporation, Deb. 2 $\frac{5}{8}$ s .....	1971	402,771.92 *	336,000
250,000	Whirlpool-Seeger Corp., S. F. Deb. 3 $\frac{1}{2}$ s .....	1980	250,000.00	217,500
<hr/>	<hr/>	<hr/>	<hr/>	<hr/>
\$13,199,000	Total Industrial and Miscellaneous .....		\$13,401,062.39	\$12,402,386
<hr/>	<hr/>	<hr/>	<hr/>	<hr/>
\$37,911,000	Bonds—Funds Invested .....		\$38,241,589.26	\$35,582,545
<hr/>	<hr/>	<hr/>	<hr/>	<hr/>

Number of Shares	Preferred Stocks		
1,500	Appalachian Electric Power Co., 4 $\frac{1}{2}$ % .....	\$159,000.00	\$138,000
2,000	Armstrong Cork Co., \$3.75 Cum. Pref. ....	205,500.00	162,000
1,500	Bethlehem Steel Corp., 7% Cum. Pref. ....	183,637.50	204,000
3,800	Carrier Corporation, 4 $\frac{1}{2}$ % Cum. Pref. ....	197,931.28	158,175
600	Cleveland Electric Illuminating Co., \$4.50 Cum. Pref. ....	68,112.25	56,100
1,900	Consolidated Edison Co. of N. Y., Inc., \$5.00 Cum. Pref. ....	202,815.50	186,200
775	Continental Can Co., Inc., \$3.75 Cum. Pref. ....	79,437.50	64,325
600	Corn Products Refining Co., 7% Cum. Pref. ....	110,335.18	90,000
2,075	du Pont (E. I.) de Nemours & Co., \$4.50 Cum. Pref. ....	235,401.89	205,425
1,000	El Paso Natural Gas Co., 4.10% Cum. 1st Pref. ....	111,442.21	80,000
1,500	General Motors Corp., \$5.00 Cum. Pref. ....	187,937.50	156,375
1,000	General Shoe Corporation, \$3.50 Cum. Pref. Series "A" .....	102,250.00	77,000
1,000	Grant (W. T.) Co., 3 $\frac{3}{4}$ % Cum. Pref. ....	100,447.91	78,000
800	National Distillers & Chemical Corp., 4 $\frac{1}{4}$ % Cum. Conv. Pref. ....	80,000.00	70,400
2,000	Niagara Mohawk Power Corp., 3.60% Cum. Pref. ....	207,990.00	140,000
1,300	Ohio Power Co., 4 $\frac{1}{2}$ % Cum. Pref. ....	144,630.02	118,950
1,500	Pacific Telephone and Telegraph Co., 6% Cum. Pref. ....	235,220.75	190,500
1,000	Panhandle Eastern Pipe Line Co., 4% Cum. Pref. ....	104,166.68	86,000
673	Pillsbury Mills, Inc., \$4.00 Cum. Pref. ....	72,496.91	59,897
2,000	Reynolds (R. J.) Tobacco Co., 3.60% Cum. Pref. ....	199,683.75	147,000
3,100	United States Steel Corp., 7% Cum. Pref. ....	443,407.57	428,575
<hr/>	<hr/>	<hr/>	<hr/>
31,623	Total Preferred Stocks .....	\$3,431,844.40	\$2,896,922
<hr/>	<hr/>	<hr/>	<hr/>

	Common Stocks		
22,500	Aluminium Limited .....	\$453,745.79	\$1,085,625
3,000	Aluminum Company of America .....	125,062.50	286,500
15,682	American Gas and Electric Company .....	214,287.05	570,433
5,500	American Telephone & Telegraph Co. ....	787,124.15	955,625
33	Applied Science Corporation of Princeton .....	1,039.60	1,716
6,000	Armco Steel Corporation .....	225,533.71	344,250
14,100	Armstrong Cork Company .....	231,516.80	377,175
10,000	Atchison, Topeka and Santa Fe Rwy. Co. ....	166,256.21	240,000
16,000	Bethlehem Steel Corp. ....	297,908.79	780,000
4,000	Caterpillar Tractor Company .....	96,913.60	362,000
3,000	Central & Southwest Corporation .....	110,250.00	112,875
2,708	Chase Manhattan Bank of New York .....	81,137.04	128,292
60	Christiana Securities Co. ....	356,143.00	786,000

\*After deduction for amortization of premiums on bonds purchased subsequent to January 1, 1940.



SCHEDULE OF SECURITIES—Continued

Number of Shares	Description	Book Value	Approximate Market Value
<u>Common Stocks—Concluded</u>			
4,200	Consumers Power Co. ....	\$145,974.04	\$190,050
4,600	Continental Can Company, Inc. ....	106,779.99	215,050
24,400	Continental Oil Co. of Delaware ....	239,598.64	1,586,000
2,500	Corning Glass Works ....	59,631.83	233,750
4,369	Dow Chemical Company ....	154,107.31	282,347
3,800	du Pont (E. I.) de Nemours & Co. ....	155,091.50	731,500
8,797	Eastman Kodak Company ....	209,365.98	969,869
5,700	First National City Bank of New York ....	279,775.25	349,838
6,000	Florida Power & Light Company ....	148,863.69	291,000
2,500	Ford Motor Company ....	161,250.00	136,875
34,000	General Electric Company ....	701,178.76	2,329,000
4,000	General Foods Corp. ....	83,651.42	177,000
15,000	General Motors Corporation ....	277,258.05	645,000
5,000	Goodrich (B. F.) Company ....	146,618.79	383,750
5,100	Goodyear Tire & Rubber Company of Ohio ....	323,584.14	451,350
1,800	Great Northern Paper Company ....	174,190.91	133,650
5,250	Gulf Oil Corp. ....	95,286.86	757,313
10,625	Gulf States Utilities Co. ....	223,782.34	382,500
2,500	Halliburton Oil Well Cementing Company ....	47,813.65	181,875
5,000	Illinois Power Co. ....	97,697.35	147,500
5,000	Insurance Company of North America ....	106,476.61	520,000
5,500	International Business Machines Corp. ....	203,469.61	1,820,500
5,000	International Nickel Co. of Canada, Ltd. ....	185,533.15	521,875
3,090	International Paper Company ....	135,087.31	319,429
6,500	Island Creek Coal Company ....	284,937.90	341,250
3,000	Kennecott Copper Corporation ....	153,174.73	329,625
8,640	Kimberly-Clark Corporation ....	182,251.11	423,360
9,400	Lehigh Portland Cement Company ....	278,294.96	344,275
1,280	Mellon National Bank and Trust Company ....	67,193.07	143,360
5,000	Merck & Co., Inc. ....	93,798.41	186,250
9,000	Minneapolis-Honeywell Regulator Co. ....	103,971.05	958,500
12,240	Monsanto Chemical Co. ....	256,904.10	466,650
3,060	National Lead Co. ....	307,193.37	397,800
2,700	Northwest Bancorporation ....	187,854.24	178,875
3,300	Ohio Edison Co. ....	105,150.00	159,225
8,000	Panhandle Eastern Pipe Line Co. ....	431,553.54	412,000
2,400	Phelps Dodge Corporation ....	71,057.69	127,800
1,990	Pittsburgh Plate Glass Co. ....	61,151.17	158,454
8,400	Procter & Gamble Co. ....	177,227.28	396,900
15,000	Puget Sound Power and Light Company ....	367,935.80	403,125
4,000	Scott Paper Co. ....	53,041.98	243,000
5,300	Seaboard Oil Co. ....	155,673.38	401,475
12,342	Shell Oil Company ....	413,016.26	1,064,498
12,375	Socony Mobil Oil Company, Inc. ....	300,464.13	745,594
5,800	Southern California Edison Company ....	208,276.33	280,575
11,250	Southern Railway Co. ....	218,508.81	473,906
8,000	Standard Oil Co. of Indiana ....	145,038.34	423,000
30,288	Standard Oil Co. of New Jersey ....	256,872.06	1,999,008
8,400	Texas Company ....	110,850.60	600,600
5,600	Texas Utilities Company ....	154,501.13	243,600
2,700	Union Carbide Corp. ....	84,640.89	323,325
5,000	Union Pacific R. R. Co. ....	114,547.42	143,750
8,000	United States Gypsum Co. ....	144,966.48	488,000
18,100	United States Steel Corp. ....	413,722.19	1,239,850
12,000	Virginia Electric and Power Co. ....	130,447.67	282,000
8,000	West Virginia Pulp and Paper Co. ....	226,533.72	354,000
6,800	Weyerhaeuser Timber Company ....	87,917.16	248,200
<hr/> 540,179	<hr/> Total Common Stocks .....	<hr/> \$13,957,652.39	<hr/> \$34,769,342
	Common and Preferred Stocks—Funds Invested .....	\$17,389,496.79	\$37,666,264
	Aggregate Investments (Bonds and Stocks) .....	<hr/> \$55,631,086.05	<hr/> \$73,248,809

SCHEDULE OF SECURITIES—Concluded

SUMMARY OF SECURITY TRANSACTIONS JULY 1, 1956 TO JUNE 30, 1957

Cash awaiting investment—July 1, 1956 .....	\$94,434.93
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Sales and Redemptions

	Gain	Loss	Book Value	
Bonds .....		\$414,282.35	\$9,988,721.08	
Preferred Stocks .....		31,957.54	333,456.35	
Common Stocks .....	\$2,680,430.32	.....	2,061,163.31	
Sale of Stock Rights .....	14,033.94	.....	.....	
	<u>\$2,694,464.26</u>	<u>\$446,239.89</u>	<u>\$12,383,340.74</u>	
Net Gain—To Exhibit E .....	.....	\$2,248,224.37	2,248,224.37	\$14,631,565.11
	<u><u>\$2,694,464.26</u></u>	<u><u>\$2,694,464.26</u></u>		

Income applied to amortization of bond premiums .....	24,062.57
Gift of common stock .....	1,024.00
Market value of stock dividend .....	7,210.88
Surplus cash transferred for investment .....	<u>400,000.00</u>
Total .....	\$15,158,297.49

Acquisitions

Bonds .....	\$13,872,758.53	
Common Stocks .....	<u>1,233,688.96</u>	<u>15,106,447.49</u>
Cash awaiting investment—June 30, 1957 .....		<u><u>\$51,850.00</u></u>



## SCHEDULE 3

SUMMARY OF CHANGES IN CURRENT FUNDS SURPLUS ACCOUNTS AND RESTRICTED GIFTS AND GRANTS  
FOR THE YEAR ENDED JUNE 30, 1957

	Balance June 30, 1956	Additions			Deductions			Balance June 30, 1957
		Trustees' Appropriations (Schedule 4)	Allotments and Transfers—Net	Other Credits	Transfers from Special Funds	Expenditures (Schedule 4)	Transfers to General Contingent Fund (See Note)	
Current Funds Surplus:								
Appropriated:								
Departmental Research Operations:								
Department of Plant Biology .....	\$4,013.15	\$91,700.00	\$9,831.46	.....	.....	\$84,138.97	\$19,138.21	\$2,267.43
Department of Genetics .....	1,615.38	173,850.00	6,973.66	.....	.....	175,351.10	1,138.22	5,949.72
Dormitory and Mess Hall .....	1,859.15	1,200.00	.....	\$10,859.71	.....	10,418.43	.....	3,500.43
Geophysical Laboratory .....	23,499.93	230,000.00	45,205.74	3,056.11	.....	266,306.11	6,235.60	29,220.07
Department of Archaeology .....	1,908.96	71,650.00	.....	.....	.....	55,307.80	15,601.52	2,649.64
Mount Wilson Observatory .....	26,756.22	296,500.00	2,000.00	.....	.....	274,918.42	24,395.44	25,942.36
Department of Terrestrial Magnetism .....	23,754.87	329,970.00	20,987.26	.....	.....	344,109.76	12,451.91	18,150.46
Department of Embryology .....	24,133.69	145,190.00	8,000.00	.....	.....	162,102.58	4,625.00	10,596.11
Total Departmental Research Operations .....	\$107,541.35	\$1,340,060.00	\$92,998.12	\$13,915.82	.....	\$1,372,653.17	\$83,585.90	\$98,276.22
Administration .....	5,291.51	295,450.00	35,598.79	.....	.....	327,382.41	840.40	8,117.49
General Operations .....	69,979.59	198,950.00	-223,240.43	.....	-\$5,079.27	.....	.....	40,609.89
General Publications .....	69,873.45	15,000.00	-500.00	6,456.03	.....	29,344.92	.....	61,484.56
Research Projects, Fellowships, etc. ....	109,749.38	.....	200,514.90	2,311.66	105,000.00	171,606.83	.....	245,969.11
Hospitalization Plan .....	.....	.....	24,000.00	.....	.....	15,939.92	.....	8,060.08
Pension Fund .....	127,554.83	80,250.00	4,730.54	.....	.....	95,138.38	.....	117,396.99
Retirement Plan Contributions .....	4,738.21	146,500.00	39,210.48	.....	.....	177,760.08	.....	12,688.61
Total .....	\$494,728.32	\$2,076,210.00	\$173,312.40	\$22,683.51	\$99,920.73	\$2,189,825.71	\$84,426.30	\$592,602.95
Unallotted:								
General Contingent Fund .....	205,982.72	.....	-173,312.40	.....	-6,583.58	.....	-84,426.30	110,513.04
Total Current Funds Surplus— Exhibit C .....	\$700,711.04	\$2,076,210.00	.....	\$22,683.51	\$93,337.15	\$2,189,825.71	.....	\$703,115.99
Restricted Gifts and Grants—Exhibit D .....	63,070.09	.....	.....	162,687.00	.....	91,165.91	.....	134,591.18
Total .....	\$763,781.13	\$2,076,210.00	.....	\$185,370.51	\$93,337.15	\$2,280,991.62	.....	\$837,707.17
Note: Transfers to General Contingent Fund:								
From Current Year's Appropriations—Schedule 4 .....								\$82,474.08
From Prior Years' Appropriations .....								1,952.22
Total .....								\$84,426.30

SCHEDULE 4  
STATEMENT OF EXPENDITURES AND BUDGET SUMMARY INCLUDING MISCELLANEOUS RECEIPTS  
FOR THE YEAR ENDED JUNE 30, 1957

FOR THE YEAR ENDED JUNE 30, 1957

Expenditures Against							
	Salaries	Fellow- ships	Equip- ment (Exhibit F)	Other Expenses	Total Expendi- tures	Current Year's Appropriations and Other Credits	Prior Years' Appro- priations
Departmental Research Operations:							
Department of Plant Biology	\$55,878.75	.....	\$8,381.59	\$19,878.63	\$84,138.97	\$74,555.10	\$9,583.87
Department of Genetics	135,929.35	.....	6,308.06	33,113.69	175,351.10	172,566.47	2,784.63
Dormitory and Mess Hall	3,793.00	.....	.....	6,625.43	10,418.43	8,559.28	1,859.15
Geophysical Laboratory	166,040.10	.....	8,128.13	92,137.88	266,306.11	235,629.38	30,676.73
Department of Archaeology	44,611.17	.....	.....	10,696.63	55,307.80	53,398.84	1,908.96
Mount Wilson Observatory	213,553.18	.....	6,468.85	54,896.39	274,918.42	247,212.20	27,706.22
Department of Terrestrial Magnetism	243,939.31	.....	20,443.61	79,726.84	344,109.76	324,989.72	19,120.04
Department of Embryology	105,765.00	.....	24,231.37	32,106.21	162,102.58	136,104.39	25,998.19
Total Departmental Research Operations	\$969,509.86	.....	\$73,961.61	\$329,181.70	\$1,372,653.17	\$1,253,015.38	\$119,637.79
Administration							
General Publications	208,045.31	.....	.....	119,337.10	327,382.41	309,681.92	17,700.49
Research Projects, Fellowships, etc.	.....	.....	.....	29,344.92	29,344.92	.....	29,344.92
Hospitalization Plan	622.50	\$78,912.23	13,752.81	78,319.29	171,606.83	25,301.68	146,305.15
Pension Fund	.....	.....	.....	15,939.92	15,939.92	15,939.92	.....
Retirement Plan Contributions	.....	.....	.....	95,138.38	95,138.38	80,250.00	14,888.38
Total	\$1,178,177.67	\$78,912.23	\$87,714.42	\$845,021.39	\$2,189,825.71	\$1,822,738.50	\$367,087.21
Restricted Gifts and Grants (see contra income—Exhibit B)							
Total	32,549.44	17,700.00	.....	40,916.47	91,165.91	.....	.....
Total	\$1,210,727.11	\$96,612.23	\$87,714.42	\$885,937.86	\$2,280,991.62	.....	.....
Budget Summary							
Budgets approved by Trustees:							
Appropriations July 1 to December 31, 1956	.....	.....	.....	\$994,715.00	.....	.....	.....
Appropriations January 1 to June 30, 1957	.....	.....	.....	1,081,495.00	\$2,076,210.00	.....	.....
Total Appropriations—Schedule 3							
Other Credits—Exhibit B:							
Sales of publications	.....	.....	.....	\$6,456.03	.....	.....	.....
Proceeds from Dormitory and Mess Hall, Cold Spring Harbor	.....	.....	.....	10,859.71	22,683.51	.....	.....
Miscellaneous	.....	.....	.....	5,367.77	.....	.....	.....
Transfer of unexpended current appropriations to General Contingent Fund—Exhibit B							
Reserved from this year's appropriations for current liabilities and commitments—Exhibit B	.....	.....	.....	.....	.....	82,474.08	.....
Income added to Special Funds—Exhibit B	.....	.....	.....	.....	.....	188,601.66	.....
Total	.....	.....	.....	.....	\$2,098,893.51	5,079.27	\$2,098,893.51





## ABSTRACT OF MINUTES OF THE FIFTY-NINTH MEETING OF THE BOARD OF TRUSTEES

The annual meeting of the Board of Trustees was held in New York City on Thursday, October 24, 1957. The Chairman, Mr. Gifford, presided.

The following Trustees were in attendance: Robert Woods Bliss, Lindsay Bradford, Walter S. Gifford, Caryl P. Haskins, Barklie McKee Henry, Robert A. Lovett, Keith S. McHugh, Margaret Carnegie Miller, Henry S. Morgan, Henning W. Prentis, Jr., Elihu Root, Jr., Henry R. Shepley, and James N. White.

The minutes of the fifty-eighth meeting were approved.

With unanimous consent Seeley G. Mudd was re-elected a member of the Board of Trustees.

The report of the President was accepted.

The report of the Executive Committee was accepted.

Article III, Section 2, and Article IV, Section 1, of the By-Laws of the Institution were amended to read as follows:

### ARTICLE III

#### *Executive Administration*

##### *The President*

2. He shall be the legal custodian of the seal and of all property of the Institution whose custody is not otherwise provided for. He shall sign and execute on behalf of the corporation all contracts and instruments necessary in authorized administrative and research matters and affix the corporate seal thereto when necessary, and may delegate the performance of such acts and other administrative duties in his absence to the Executive Officer. He may execute all other contracts, deeds, and instruments on behalf of the corporation and affix the seal thereto when expressly authorized by the Board of Trustees or Executive Committee. He may, within the limits of his own authorization, delegate to the Executive Officer authority to act as custodian of and affix the corporate seal. He shall be responsible for the expenditure and disbursement of all funds of the Institution in accordance with the directions of the Board and of the Executive Committee, and shall keep accurate accounts of all receipts and disbursements. Following approval by the Executive Committee he shall transmit to the Board of Trustees before its annual meeting a written report of the operations and business of the Institution for the preceding fiscal year with his recommendations for work and appropriations for the succeeding fiscal year.

### ARTICLE IV

#### *Meetings*

1. The annual meeting of the Board of Trustees shall be held in the City of Washington, in the District of Columbia, on the second Friday of May in each year, unless the date and place of meeting are otherwise set by order of the Executive Committee.



To provide for operation of the Institution from January 1, 1958, to June 30, 1958, and upon recommendation of the Executive Committee, the sum of \$1,112,100.00 was appropriated from the General Reserve Fund.

The sum of \$287,900.00 was appropriated from the General Reserve Fund and transferred to the General Contingent Fund of the Institution.

Reports of the Finance Committee, the Retirement Committee, the Auditor, and the Auditing Committee were accepted.

An existing vacancy in the membership of the Board of Trustees and the terms of Officers and Committee Members listed in the report of the Executive Committee having been considered by the Nominating Committee, the Board of Trustees accepted the Nominating Committee's recommendation that consideration of its report and elections to fill vacancies be deferred until the annual meeting of the Board on May 9, 1958.

# ARTICLES OF INCORPORATION

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*Public No. 260. An Act to incorporate the Carnegie Institution of Washington*

*Be it enacted by the Senate and House of Representatives of the United States of America in Congress assembled,* That the persons following, being persons who are now trustees of the Carnegie Institution, namely, Alexander Agassiz, John S. Billings, John L. Cadwalader, Cleveland H. Dodge, William N. Frew, Lyman J. Gage, Daniel C. Gilman, John Hay, Henry L. Higginson, William Wirt Howe, Charles L. Hutchinson, Samuel P. Langley, William Lindsay, Seth Low, Wayne MacVeagh, Darius O. Mills, S. Weir Mitchell, William W. Morrow, Ethan A. Hitchcock, Elihu Root, John C. Spooner, Andrew D. White, Charles D. Walcott, Carroll D. Wright, their associates and successors, duly chosen, are hereby incorporated and declared to be a body corporate by the name of the Carnegie Institution of Washington and by that name shall be known and have perpetual succession, with the powers, limitations, and restrictions herein contained.

*Sec. 2.* That the objects of the corporation shall be to encourage, in the broadest and most liberal manner, investigation, research, and discovery, and the application of knowledge to the improvement of mankind; and in particular—

(*a*) To conduct, endow, and assist investigation in any department of science, literature, or art, and to this end to cooperate with governments, universities, colleges, technical schools, learned societies, and individuals.

(*b*) To appoint committees of experts to direct special lines of research.

(*c*) To publish and distribute documents.

(*d*) To conduct lectures, hold meetings, and acquire and maintain a library.

(*e*) To purchase such property, real or personal, and construct such building or buildings as may be necessary to carry on the work of the corporation.

(*f*) In general, to do and perform all things necessary to promote the objects of the institution, with full power, however, to the trustees hereinafter appointed and their successors from time to time to modify the conditions and regulations under which the work shall be carried on, so as to secure the application of the funds in the manner best adapted to the conditions of the time, provided that the objects of the corporation shall at all times be among the foregoing or kindred thereto.

*Sec. 3.* That the direction and management of the affairs of the corporation and the control and disposal of its property and funds shall be vested in a board of trustees, twenty-two in number, to be composed of the following individuals: Alexander Agassiz, John S. Billings, John L. Cadwalader, Cleveland H. Dodge, William N. Frew, Lyman J. Gage, Daniel C. Gilman, John Hay, Henry L. Higginson, William Wirt Howe, Charles L. Hutchinson, *Samuel P. Langley*, William Lindsay, Seth Low, Wayne MacVeagh, Darius O. Mills, S. Weir Mitchell, William W. Morrow, *Ethan A. Hitchcock*, Elihu Root, John C. Spooner, Andrew D. White, Charles D. Walcott, Carroll D. Wright, who shall constitute



the first board of trustees. The board of trustees shall have power from time to time to increase its membership to not more than twenty-seven members. Vacancies occasioned by death, resignation, or otherwise shall be filled by the remaining trustees in such manner as the by-laws shall prescribe; and the persons so elected shall thereupon become trustees and also members of the said corporation. The principal place of business of the said corporation shall be the city of Washington, in the District of Columbia.

*Sec. 4.* That such board of trustees shall be entitled to take, hold, and administer the securities, funds, and property so transferred by said Andrew Carnegie to the trustees of the Carnegie Institution and such other funds or property as may at any time be given, devised, or bequeathed to them, or to such corporation, for the purposes of the trust; and with full power from time to time to adopt a common seal, to appoint such officers, members of the board of trustees or otherwise, and such employees as may be deemed necessary in carrying on the business of the corporation, at such salaries or with such remuneration as they may deem proper; and with full power to adopt by-laws from time to time and such rules or regulations as may be necessary to secure the safe and convenient transaction of the business of the corporation; and with full power and discretion to deal with and expend the income of the corporation in such manner as in their judgment will best promote the objects herein set forth and in general to have and use all powers and authority necessary to promote such objects and carry out the purposes of the donor. The said trustees shall have further power from time to time to hold as investments the securities hereinabove referred to so transferred by Andrew Carnegie, and any property which has been or may be transferred to them or such corporation by Andrew Carnegie or by any other person, persons, or corporation, and to invest any sums or amounts from time to time in such securities and such form and manner as are permitted to trustees or to charitable or literary corporations for investment, according to the laws of the States of New York, Pennsylvania, or Massachusetts, or in such securities as are authorized for investment by the said deed of trust so executed by Andrew Carnegie, or by any deed of gift or last will and testament to be hereafter made or executed.

*Sec. 5.* That the said corporation may take and hold any additional donations, grants, devises, or bequests which may be made in further support of the purposes of the said corporation, and may include in the expenses thereof the personal expenses which the trustees may incur in attending meetings or otherwise in carrying out the business of the trust, but the services of the trustees as such shall be gratuitous.

*Sec. 6.* That as soon as may be possible after the passage of this Act a meeting of the trustees hereinbefore named shall be called by Daniel C. Gilman, John S. Billings, Charles D. Walcott, S. Weir Mitchell, John Hay, Elihu Root, and Carroll D. Wright, or any four of them, at the city of Washington, in the District of Columbia, by notice served in person or by mail addressed to each trustee at his place of residence; and the said trustees, or a majority thereof, being assembled, shall organize and proceed to adopt by-laws, to elect officers and appoint committees, and generally to organize the said corporation; and said trustees herein named, on behalf of the corporation hereby incorporated, shall thereupon receive, take over, and enter into possession, custody, and management of all property, real or personal, of the corporation heretofore known as the Carnegie Institution, incorporated, as hereinbefore set forth under "An Act to establish a Code of Law for the District of Columbia, January fourth, nineteen hundred and two," and to all its rights, contracts,

claims, and property of any kind or nature; and the several officers of such corporation, or any other person having charge of any of the securities, funds, real or personal, books, or property thereof, shall, on demand, deliver the same to the said trustees appointed by this Act or to the persons appointed by them to receive the same; and the trustees of the existing corporation and the trustees herein named shall and may take such other steps as shall be necessary to carry out the purposes of this Act.

*Sec. 7.* That the rights of the creditors of the said existing corporation known as the Carnegie Institution shall not in any manner be impaired by the passage of this Act, or the transfer of the property hereinbefore mentioned, nor shall any liability or obligation for the payment of any sums due or to become due, or any claim or demand, in any manner or for any cause existing against the said existing corporation, be released or impaired; but such corporation hereby incorporated is declared to succeed to the obligations and liabilities and to be held liable to pay and discharge all of the debts, liabilities, and contracts of the said corporation so existing to the same effect as if such new corporation had itself incurred the obligation or liability to pay such debt or damages, and no such action or proceeding before any court or tribunal shall be deemed to have abated or been discontinued by reason of the passage of this Act.

*Sec. 8.* That Congress may from time to time alter, repeal, or modify this Act of incorporation, but no contract or individual right made or acquired shall thereby be divested or impaired.

*Sec. 9.* That this Act shall take effect immediately.

*Approved, April 28, 1904*





# BY-LAWS OF THE INSTITUTION

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*Adopted December 13, 1904. Amended December 13, 1910, December 13, 1912, December 10, 1937, December 15, 1939, December 13, 1940, December 18, 1942, December 12, 1947, December 10, 1954, and October 24, 1957*

## ARTICLE I

### *The Trustees*

1. The Board of Trustees shall consist of twenty-four members, with power to increase its membership to not more than twenty-seven members. The Trustees shall hold office continuously and not for a stated term.

2. In case any Trustee shall fail to attend three successive annual meetings of the Board he shall thereupon cease to be a Trustee.

3. No Trustee shall receive any compensation for his services as such.

4. All vacancies in the Board of Trustees shall be filled by the Trustees by ballot at an annual meeting, but no person shall be declared elected unless he receives the votes of two-thirds of the Trustees present.

## ARTICLE II

### *Officers of the Board*

1. The officers of the Board shall be a Chairman of the Board, a Vice-Chairman, and a Secretary, who shall be elected by the Trustees, from the members of the Board, by ballot to serve for a term of three years. All vacancies shall be filled by the Board for the unexpired term; provided, however, that the Executive Committee shall have power to fill a vacancy in the office of Secretary to serve until the next meeting of the Board of Trustees.

2. The Chairman shall preside at all meetings and shall have the usual powers of a presiding officer.

3. The Vice-Chairman, in the absence or disability of the Chairman, shall perform the duties of the Chairman.

4. The Secretary shall issue notices of meetings of the Board, record its transactions, and conduct that part of the correspondence relating to the Board and to his duties.

## ARTICLE III

### *Executive Administration*

#### *The President*

1. There shall be a President who shall be elected by ballot by, and hold office during the pleasure of, the Board, who shall be the chief executive officer of the Institution. The President, subject to the control of the Board and the Executive Committee, shall have



general charge of all matters of administration and supervision of all arrangements for research and other work undertaken by the Institution or with its funds. He shall prepare and submit to the Board of Trustees and to the Executive Committee plans and suggestions for the work of the Institution, shall conduct its general correspondence and the correspondence with applicants for grants and with the special advisers of the Committee, and shall present his recommendations in each case to the Executive Committee for decision. All proposals and requests for grants shall be referred to the President for consideration and report. He shall have power to remove, appoint, and, within the scope of funds made available by the Trustees, provide for compensation of subordinate employees and to fix the compensation of such employees within the limits of a maximum rate of compensation to be established from time to time by the Executive Committee. He shall be *ex officio* a member of the Executive Committee.

2. He shall be the legal custodian of the seal and of all property of the Institution whose custody is not otherwise provided for. He shall sign and execute on behalf of the corporation all contracts and instruments necessary in authorized administrative and research matters and affix the corporate seal thereto when necessary, and may delegate the performance of such acts and other administrative duties in his absence to the Executive Officer. He may execute all other contracts, deeds, and instruments on behalf of the corporation and affix the seal thereto when expressly authorized by the Board of Trustees or Executive Committee. He may, within the limits of his own authorization, delegate to the Executive Officer authority to act as custodian of and affix the corporate seal. He shall be responsible for the expenditure and disbursement of all funds of the Institution in accordance with the directions of the Board and of the Executive Committee, and shall keep accurate accounts of all receipts and disbursements. Following approval by the Executive Committee he shall transmit to the Board of Trustees before its annual meeting a written report of the operations and business of the Institution for the preceding fiscal year with his recommendations for work and appropriations for the succeeding fiscal year.

3. He shall attend all meetings of the Board of Trustees.

4. There shall be an officer designated Executive Officer who shall be appointed by and hold office at the pleasure of the President, subject to the approval of the Executive Committee. His duties shall be to assist and act for the President as the latter may duly authorize and direct.

5. The President shall retire from office at the end of the calendar year in which he becomes sixty-five years of age.

#### ARTICLE IV

##### *Meetings*

1. The annual meeting of the Board of Trustees shall be held in the City of Washington, in the District of Columbia, on the second Friday of May in each year, unless the date and place of meeting are otherwise set by order of the Executive Committee.

2. Special meetings of the Board may be called by the Executive Committee by notice served personally upon, or mailed to the usual address of, each Trustee twenty days prior to the meeting.

3. Special meetings shall, moreover, be called in the same manner by the Chairman upon the written request of seven members of the Board.

## ARTICLE V

*Committees*

1. There shall be the following standing Committees, *viz.* an Executive Committee, a Finance Committee, an Auditing Committee, a Nominating Committee, and a Retirement Committee.

2. All vacancies occurring in the Executive Committee, the Finance Committee, the Auditing Committee, the Nominating Committee, and the Retirement Committee shall be filled by the Trustees at the next regular meeting. In case of vacancy in the Finance Committee, the Auditing Committee, the Nominating Committee, or the Retirement Committee, upon request of the remaining members of such committee, the Executive Committee may fill such vacancy by appointment until the next meeting of the Board of Trustees.

3. The terms of all officers and of all members of committees, as provided for herein, shall continue until their successors are elected or appointed.

*Executive Committee*

4. The Executive Committee shall consist of the Chairman, Vice-Chairman, and Secretary of the Board of Trustees and the President of the Institution *ex officio* and, in addition, five trustees to be elected by the Board by ballot for a term of three years, who shall be eligible for re-election. Any member elected to fill a vacancy shall serve for the remainder of his predecessor's term.

5. The Executive Committee shall, when the Board is not in session and has not given specific directions, have general control of the administration of the affairs of the corporation and general supervision of all arrangements for administration, research, and other matters undertaken or promoted by the Institution. It shall also submit to the Board of Trustees a printed or typewritten report of each of its meetings, and at the annual meeting shall submit to the Board a report for publication.

6. The Executive Committee shall have power to authorize the purchase, sale, exchange, or transfer of real estate.

*Finance Committee*

7. The Finance Committee shall consist of not less than five and not more than six members to be elected by the Board of Trustees by ballot for a term of three years, who shall be eligible for re-election.

8. The Finance Committee shall have custody of the securities of the corporation and general charge of its investments and invested funds, including its investments and invested funds as trustee of any retirement plan for the Institution's staff members and employees, and shall care for and dispose of the same subject to the directions of the Board of Trustees. It shall have power to authorize the purchase, sale, exchange, or transfer of securities and to delegate this power. It shall consider and recommend to the Board from time to time such measures as in its opinion will promote the financial interests of the Institution and of the trust fund under any retirement plan for the Institution's staff members and employees, and shall make a report at each meeting of the Board.



*Auditing Committee*

9. The Auditing Committee shall consist of three members to be elected by the Board of Trustees by ballot for a term of three years.

10. Before each annual meeting of the Board of Trustees, the Auditing Committee shall cause the accounts of the Institution for the preceding fiscal year to be audited by public accountants. The accountants shall report to the Committee, and the Committee shall present said report at the ensuing annual meeting of the Board with such recommendations as the Committee may deem appropriate.

*Nominating Committee*

11. The Nominating Committee shall consist of the Chairman of the Board of Trustees *ex officio* and, in addition, three trustees to be elected by the Board by ballot for a term of three years, who shall not be eligible for re-election until after the lapse of one year. Any member elected to fill a vacancy shall serve for the remainder of his predecessor's term, provided that of the Nominating Committee first elected after adoption of this By-Law one member shall serve for one year, one member shall serve for two years, and one member shall serve for three years, the Committee to determine the respective terms by lot.

12. Sixty days prior to an annual meeting of the Board the Nominating Committee shall notify the Trustees by mail of the vacancies to be filled in membership of the Board. Each Trustee may submit nominations for such vacancies. Nominations so submitted shall be considered by the Nominating Committee, and ten days prior to the annual meeting the Nominating Committee shall submit to members of the Board by mail a list of the persons so nominated, with its recommendations for filling existing vacancies on the Board and its Standing Committees. No other nominations shall be received by the Board at the annual meeting except with the unanimous consent of the Trustees present.

*Retirement Committee*

13. The Retirement Committee shall consist of three members to be elected by the Board of Trustees by ballot for a term of three years, who shall be eligible for re-election. Any member elected to fill a vacancy shall serve for the remainder of his predecessor's term, provided that of the Retirement Committee first elected after adoption of this By-Law one member shall serve for one year, one member shall serve for two years, and one member shall serve for three years, the Committee to determine the respective terms by lot.

14. The Retirement Committee shall, subject to the directions of the Board of Trustees, be responsible for the maintenance of a retirement plan for staff members and employees of the Institution and act for the Institution in its capacity as trustee under any such plan, except that any matter relating to investments under any such plan shall be the responsibility of the Finance Committee subject to the directions of the Board of Trustees. The Committee shall submit a report to the Board at the annual meeting of the Board.

## ARTICLE VI

*Financial Administration*

1. No expenditure shall be authorized or made except in pursuance of a previous appropriation by the Board of Trustees, or as provided in Article V, paragraph 8, hereof.

2. The fiscal year of the Institution shall commence on the first day of July in each year.
3. The Executive Committee shall submit to the annual meeting of the Board a full statement of the finances and work of the Institution for the preceding fiscal year and a detailed estimate of the expenditures of the succeeding calendar year.
4. The Board of Trustees, at the annual meeting in each year, shall make general appropriations for the ensuing calendar year; but nothing contained herein shall prevent the Board of Trustees from making special appropriations at any meeting.
5. The Executive Committee shall have general charge and control of all appropriations made by the Board. Following the annual meeting each year, the Executive Committee may make allotment of funds for the period from January 1 to termination of the fiscal year on June 30. It may also make allotment of funds for the period from July 1 to December 31 in advance of July 1. The Committee shall, however, have full authority for allotment of available funds to meet necessary expenditures by other methods, if desirable, and transfer of balances to meet special needs. It shall make provision for outstanding obligations and for revertment of unexpended balances at termination of the fiscal year.
6. The securities of the Institution and evidences of property, and funds invested and to be invested, shall be deposited in such safe depository or in the custody of such trust company and under such safeguards as the Finance Committee shall designate, subject to directions of the Board of Trustees. Income of the Institution available for expenditure shall be deposited in such banks or depositories as may from time to time be designated by the Executive Committee.
7. Any trust company entrusted with the custody of securities by the Finance Committee may, by resolution of the Board of Trustees, be made Fiscal Agent of the Institution, upon an agreed compensation, for the transaction of the business coming within the authority of the Finance Committee.

## ARTICLE VII

### *Amendment of By-Laws*

1. These by-laws may be amended at any annual or special meeting of the Board of Trustees by a two-thirds vote of the members present, provided written notice of the proposed amendment shall have been served personally upon, or mailed to the usual address of, each member of the Board twenty days prior to the meeting.





















